HOSPITAL LIBRARY
JUL 29 1947

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

PAGE

THE BIOLOGICAL SIGNIFICANCE OF HYALURONIC ACID AND HYALURONIDASE. *Karl Meyer* 335

PATHOGENETIC FACTORS AND PATHOLOGICAL CONSEQUENCES OF DECOMPRESSION SICKNESS. *H. R. Catchpole and Isidore Gersh* 360

THE GENESIS OF THE ELECTROCARDIOGRAM. *Louis N. Katz* 398

INTERCELLULAR CEMENT AND CAPILLARY PERMEABILITY. *Robert Chambers and B. W. Zweifach* 436

SOME PHYSIOLOGICAL EFFECTS OF CURARE AND THEIR APPLICATION TO CLINICAL MEDICINE. *A. R. McIntyre* 464

THE URINARY COPROPORPHYRINS IN HEALTH AND DISEASE. *Cecil James Watson and Evrel A. Larson* 478

VOL. 27, NO. 3

Issued July, 1947

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the act of March 3, 1879

Made in United States of America

PHYSIOLOGICAL REVIEWS

Tentative Contents of Volume 27, 1947

B. F. MILLER: Biological Use of Detergents

E. B. ASTWOOD: Control of Thyroid Function

LOUIS N. KATZ: The Significance of the Electrocardiogram

E. J. BOELL AND J. S. NICHOLAS: Metabolism of the Embryo

F. C. MANN: Hibernation

J. H. WELSH: Acetylcholine

MCKEEN CATTELL AND HARRY GOLD: Digitalis Glycosides

ROBERT CHAMBERS AND B. W. ZWEIFACLE: Intercellular Cement and Capillary Permeability

AMEDEO S. MARRAZZI: Pharmacological Aspects of Synaptic Function

DAVID NACHMANSOHN: Cholinesterase

GEORGE WALD: The Chemistry of Visual Systems

D. B. DILL: Physiological Stresses in Aviation

HARRY GOLDBLATT: The Renal Origin of Essential Hypertension

ALLAN T. KENYON AND KATHRYN KNOWLTON: The Comparative Metabolic Properties of Certain Steroids

PHILIP BARD: Motion Sickness

FREDERICK L. HISAW: The Physiology of Ovulation

J. S. L. BROWNE AND V. SCHENKER: The Metabolic Aspects of Trauma and Convalescence

BALDUIN LUCKÉ: Neoplasia in Cold Blooded Vertebrates

H. R. CATCHPOLE AND ISIDORE GERSH: Physiological Basis of Decompression Sickness

FRANK A. BEACH: Neuro-Hormonal Factors Involved in the Mating Behavior of Animals

B. H. WILLIER: The Production of Pigmentation Patterns in Birds

HAROLD E. WOLFF: Pain

HALLOWELL DAVIS: Recent Advances in the Physiology of Audition

C. J. WATSON AND E. A. LARSON: The Urinary Coproporphyrins in Health and Disease

THOMAS F. DOUGHERTY: Hormonal Factors Affecting Lymphoid Tissue

JOHN A. LUETSCHER, JR.: Application of Electrophoresis to Medicine and Biology

A. PI-SUÑER: The Regulation of the Respiratory Movements by Peripheral Chemoreceptors

J. P. BOUCKAERT AND CHR. DE DUVE: The Action of Insulin

A. G. OGSTON: The Definition and Meaning of pH

M. S. DUNN, E. A. MURPHY AND L. B. ROCKLAND: Optimal Growth of the Rat

E. BALL AND E. A. EVANS, JR.: Biochemistry of the Malaria Parasite

KONRAD BLOCH: Intermediary Metabolism of Acetic Acid in Animal Tissues

J. L. BOLLMAN: Liver Metabolism

T. H. BULLOCK: Electrophysiology of Invertebrate Nervous Systems

THOMAS FRANCIS, JR.: Principles and Problems in Virus Infection

R. D. H. HEARD: The Origin and Significance of Urinary 17-Keto-Steroids

J. O. HUTCHENS AND M. A. LIPTON: Cyanide and Cyanogen Chloride

MAX KLEIBER: Body Size and Metabolic Rate

N. KLEITMAN: Some Physiological Rhythms

EARL R. LOEW: Pharmacology of Antihistamine Compounds

A. R. MCINTYRE: Chemical Interruption of Neuromuscular Transmission

WILLARD MACHLE AND T. F. HATCH: Adaptation of Man to Heat

KARL MEYER: The Biological Aspects of Hyaluronidase and Hyaluronic Acid

BRADLEY M. PATTEN: Initiation of Cardiac Contraction in Embryos

R. A. PETERS: The BAL Treatment of Metal Poisoning

H. P. SMITH: Blood Clotting Mechanisms

VAN S. SMITH AND O. WATKINS SMITH: Endocrines and Toxemia of Pregnancy

S. SPIEGELMAN: Mechanisms Controlling Cellular Enzymatic Constitution

A. E. WILHELMI: The Phosphatases

D. W. WOOLLEY: Competition Between Structurally Analogous Compounds in Biological Systems

E. M. LANDIS AND JOHN HORTENSTINE: The Functional Significance of Venous Blood Pressures

PHYSIOLOGICAL REVIEWS

VOL. 27 JULY, 1947 No. 3

THE BIOLOGICAL SIGNIFICANCE OF HYALURONIC ACID AND HYALURONIDASE

KARL MEYER

The Institute of Ophthalmology of Presbyterian Hospital and the College of Physicians and Surgeons of Columbia University, New York, New York

Hyaluronic acid is a mucopolysaccharide acid which in animal tissues seems to bind water in interstitial spaces. It further holds cells together in a jelly-like matrix and serves as a lubricant and shock-absorber in joints. It is desaggregated and depolymerized by the action of the enzyme hyaluronidase. In this review the effort is being made to discuss aspects most essential to an understanding of the biological rôle of hyaluronic acid and hyaluronidase.

Duran-Reynals in 1942 in a paper entitled "Tissue permeability and the spreading factors in infection" published the first comprehensive review dealing with hyaluronic acid, hyaluronidase and the spreading factors. Current literature has been briefly reviewed in 1942 (15B) and in 1944 (44A).

I. HYALURONIC ACID. A. *Occurrence.* Hyaluronic acid was first isolated from bovine vitreous humor (63). Subsequently it was isolated from human umbilical cord (64), from bovine aqueous humor and from vitreous humor of pig eyes (65), from groups A and C hemolytic streptococci (35), from bovine and human synovial fluid (66), from a number of mesenchymal tumors like fowl leucosis (33), the pleura and peritoneal fluid of a case of mesenthelioma (55), from two cases of human synovioma (unpublished), from Rous and Fuyinami tumors of chicken (71), and from pig skin (56).

Hyaluronic acid probably occurs in the nucleus pulposus of the intervertebral disc (unpublished) and in small concentrations in connective tissue, although it has not been isolated from these sources.

B. *Chemistry.* For the isolation of the acid from various sources some modifications of the same general principles have been described. From fluids like vitreous humor, synovial fluid and some tumor fluids, from which the purest samples have been obtained, the acid is first precipitated as a salt of accompanying proteins. For this purpose the fluids are diluted with 2–5 volumes of cold water and acidified to a pH of about 4 with 50 per cent acetic acid with vigorous stirring. With vitreous humor the precipitation may be incomplete until the protein content is increased by addition of horse or cattle serum. The "mucin clot" is left standing at 0° for 24 hours, is then washed with cold water and extracted with a 5 to 10 per cent solution of sodium, potassium, or calcium acetate, depending upon which salt is to be desired. The extraction may be hastened by adding alkali to a pH of 9.0.

Solid tissues like umbilical cord or some tumors are chilled, ground and desiccated with acetone. The dry material is extracted repeatedly with 5 to 10 per cent of an acetate. The crude acid is acidified to pH 4 and precipitated with 1.5

volume of ethanol. The centrifuged and washed precipitate is again extracted with acetate.

The further purification of this extract and the extracts of the protein salts described above are, from here on, identical. The bulk of the protein is removed by stirring with a chloroform-amylalcohol mixture for one hour followed by separation of the fluid from the gel by centrifugation. The procedure is repeated until a gel is no longer formed. Further nitrogenous impurities are removed either by adsorption on Zn $(OH)_2$ at pH 7.2 (adding 10 per cent zinc acetate and neutralizing) or by adsorption on Lloyd's reagent at a pH of about 4. The supernatant is precipitated with 1.5 volumes of ethanol at pH 4. The stringy mass is again centrifuged, washed with alcohol, redissolved in a minimum amount of water, precipitated by alcohol in the presence of acetate, washed with alcohol and ether and dried in vacuo over P_2O_5. In earlier preparations in this laboratory, the free acid was precipitated by pouring the aqueous solution into 6 to 10 volumes of glacial acetic acid. The products so obtained were of high purity and very low ash content (64), but were somewhat depolymerized as evidenced by a lowered viscosity and a lower optic rotation.

The hyaluronate preparations sometimes contain nitrogenous impurities, glycogen, and organic and inorganic sulfate. Nitrogenous impurities shown by an increased ratio of total N to hexosamine N can be removed by repeated adsorption. Glycogen, present especially in skin and umbilical cord preparations, is indicated by too low analytical figures (see below) and is removed by incubation for a few hours with either filtered saliva or a commercial amylase. Sulfates are difficult to remove, especially in umbilical cord hyaluronate. The S content may be lowered by dissolving the purified material in 10 per cent calcium acetate or chloride and fractionating with 25 to 33 per cent ethanol. After the mixture has stood at 0° for 24 hours the bulk of the sulfate is found in the precipitate.

Several authors have reported simplified methods for the preparation of hyaluronate. One such method (18) yielded a preparation which after removal of some insoluble material contained only 16 per cent of standard hyaluronate.

The preparations in this laboratory are analyzed for N by the Kjeldahl method, for hexosamine, for acetyl by the Kuhn-Roth method, for uronic acid by the Freudenberg modification of the Tollens-Lefevre method, and for ash.

The rotation $[\alpha]_D^{20}$ of the sodium salt is $-70°$, that of the free acid usually is somewhat lower, depending on the time of contact with acetic acid. The viscosity relative to 0.9 per cent sodium chloride at 37° of a 0.3 per cent solution of the sodium salt usually is approximately 4, and varies with the source. From vitreous humor by fractionation with $(NH_4)_2SO_4$ at a pH of about 9.0, fractions of widely varying viscosities were obtained. This is probably due to enzymatic depolymerization in vivo. The acid is poly-disperse in other sources as well, as pointed out by Blix and Snellman (4), who measured viscosities and streaming double refraction of flow. The latter was positive for both hyaluronate and chondroitin sulfate of cartilage. The particle length of different preparations of hyaluronate varied between 4800A° and 10,000A° with the higher values

in preparations of umbilical cord. The corresponding molecular weights were estimated to be in the order of 200,000 to 500,000 with the assumption of an unbranched chain and a length of 10A° of the basic disaccharide unit. The molecular weights, according to the authors, should be regarded as minimal values.

X-ray measurements of sodium hyaluronate gave only diffuse patterns in powder and fibre diagrams.

The viscosity of isolated hyaluronate is much smaller than that of the fluids from which it is obtained. Whether this is due to oxidative breakdown, as suggested by some authors (83), or to another process of disaggregation or depolymerization cannot be decided with certainty. In the native state hyaluronate forms gels of varying rigidity. For example a cyst formed by an undiagnosed malignant tumor of the bone had the consistency of a 4 per cent agar solution. The cyst content was liquefied rapidly by incubation with testicular hyaluronidase. Samples of human synovial fluid exhibit viscosities ranging from very low values to high viscosities which are outside the range of our viscosimeters. The dilution curve of the tumor fluids showed a sharp drop in viscosity with dilution (55) and the same has been shown by Ragan (72) for normal human joint fluid. Obviously the viscosity in these fluids is of a non-Newtonian type. On the other hand, isolated hyaluronate has all the characteristics of a highly asymmetrical molecule. Aqueous solutions have high viscosity, they possess bi-refringence of flow, and can be spun into threads of considerable tensile strength.

The difference between the viscosity of the native fluid and that of isolated hyaluronate may be due to complex formation with accompanying protein, but there is no experimental proof for this. The mobility of isolated hyaluronate in the Tiselius apparatus at pH 7.8 was identical with that of the fast component in tumor and synovial fluids (55, 3). No fraction with a mobility intermediate between protein and polysaccharide was observed. Furthermore, on incubation of the native fluids with hyaluronidase, the viscosities drop rapidly to the values for the proteins present. It is concluded from these experiments that hyaluronate exists in these fluids in varying degree of polymerization and aggregation, the latter being due to weak secondary bonds either between the polysaccharide molecules themselves or between protein and polysaccharide. The weak bonds are broken by the processes involved in the isolation.

The chemical structure of hyaluronic acid is unknown. Equimolar quantities of hexosamine, acetyl and uronic acid have been found by analysis. Glucosamine has been isolated (as the crystalline hydrochloride) in yields up to 95 per cent of the colorimetrically determined amount. The presence of glucuronic acid was established by oxidation of hyaluronic acid by HNO_3 and isolation of saccharic acid as the crystalline acid potassium salt. From an enzymatic digest of synovial hyaluronate with pneumococcus hyaluronidase, glucuronic acid has been isolated as thiosemicarbazone (66). Hyaluronic acid, in contrast to chondroitin sulfate, probably contains little or no branched chains. This is indicated by the almost quantitative hydrolysis by some hyaluronidase preparations. It can be deduced from enzymatic data (see later) that the

basic unit is a disaccharide with a free aldehyde group present in the acetylglucosamine moiety.

The occurrence of ester linkages in the hyaluronate chains has been recently suggested (4), on the basis of the alkali sensitivity of the polysaccharide. In 4–5 days in absence of O_2 at 20° about one milliequivalent of acid was produced for about every 4 disaccharide units. Apparently the reaction came to about the same endpoint with different samples of the polysaccharide and with varying alkali concentrations. About 10 per cent of the weight of the polysaccharide dialyzed through a cellophane membrane and the rest was clearly not of uniform size. The analysis for sodium in the neutral native material did not support the presence of ester linkages. Sensitivity to alkali with production of acidic groups with fission of the chains is common to many other polysaccharides, for example cellulose, though the mechanism is not clearly understood.

Hyaluronate on injection into animals is not antigenic. Attempts to confer antigenicity to hyaluronate have been unsuccessful (29).

II. HYALURONIDASE. A. *Occurrence.* The enzyme hydrolyzing hyaluronic acid, hyaluronidase, was first found in autolysates of a rough type II pneumococcus (58). Its occurrence was shown further in other types of pneumocccci both smooth and rough, in strains of hemolytic streptococci of groups A and C, and in some anaerobes (62). Its occurrence in the animal body in extracts of spleen and of ciliary body and iris, has been reported (62).

The very wide distribution of hyaluronidase, however, was found after Chain and Duthie (8) discovered that the hyaluronidase activity of testicular extracts paralleled the activity of the "spreading factor", reported earlier by Duran-Reynals (27) and by McClean (46). Hyaluronidase activity, as measured by the hydrolysis or depolymerization of hyaluronic acid and hyaluronate-containing fluids, was then demonstrated in virulent and avirulent strains of type I pneumococcus (57), in a group C hemolytic streptococcus and in rabbit skin (ibid), in the venoms of many poisonous snakes, like copperhead, Black Tiger (9), rattlesnake (15), in bee venom (9), in Staph. aureus, Cl. Welchii, (9), and others.

B. *Determination of Hyaluronidase.* For the determination of hyaluronidase biological, chemical and physico-chemical methods have been used.

1. *Biological methods.* The spreading reaction has been reviewed by Duran-Reynals (12) and need be mentioned here only briefly. India ink probably is the best indicator, since with it the area of spreading is more circumscribed than with diffusible dyes. The most convenient dye is the blue dye T1824, since it is non-toxic and available in sterilized isotonic solution. An improved quantitative method for the estimation of the spreading reaction has been published by Humphrey (28) who injected the spreading agent intracutaneously into groups of 6 guinea pigs. The animals were killed 20 minutes after injection, the skin was removed immediately and the size of the bleb measured at the inner surface of the skin. The minimal diffusion dose is the least amount of enzyme which will produce a 20 per cent increase of the mean area over the mean area of the controls.

The spreading reaction cannot be considered as an accurate assay of hyaluronidase. Some unspecific agents give spreading reactions. Furthermore, the correlation between spreading reaction and physico-chemical methods of hyaluronidase estimation is poor. (See later.) The influence of pressure on the assay of hyaluronidase by the spreading reaction has been stressed in a recent article (23A). Most investigators however have in the past used a constant and small volume of 0.1 or 0.2 cc. Control injections with identical volumes have probably been used by all investigators. Humphrey has emphasized the importance of the rate of the initial reaction by using a reaction time of only 20 minutes.

The decapsulation of mucoid hemolytic streptococci of groups A and C has been compared with hyaluronidase activity estimated by other methods (47). However, these organisms lose their capsules under various conditions without added enzyme. It is not known whether or not this is due to hyaluronidase produced by the same cultures (see later). The decapsulation of streptococci can hardly be considered as a convenient method for hyaluronidase estimation.

2. *Physico-chemical methods.* Three methods will be discussed: *a*, the mucin clot prevention test (abbreviated M.C.P.); *b*, the viscosity reducing method, and *c*, the turbidimetric method.

a. This method is based on the observation that native hyaluronic acid in acid solution coprecipitates with protein in a typical fibrous "mucin" clot. After incubation with hyaluronidase the quantity of the clot is reduced and the character of the precipitate changes from a fibrous to a flocculent precipitate, until finally the solutions remain clear. The test was apparently first used by Robertson, Ropes and Bauer (76) and has been modified by McClean and his collaborators (52).

The test is simple and suited for serial determinations and is probably the most sensitive test for low concentrations of hyaluronidase. However, it is positive only with crude hyaluronate, the mechanism of its action is not clear and it has a very poor correlation with other tests. The M.C.P. test is carried out in the native fluid or the isolated and redissolved protein salt. Pure hyaluronate precipitates protein in acid solution in flocculent form. (The difference between native and isolated hyaluronate has been discussed on p. 337.)

The error of the M.C.P test has been estimated (48) as ± 25 per cent. The author found no correlation between M.C.P. units and viscosity reducing units, nor a constant trend with the potency or the source of the enzyme. Similarly in another paper from the same laboratory (77) the ratios of M.C.P. over viscosity reducing units with different micro-organisms varied between 1:20 to 1:162. The reason for the discrepancy between the two tests cannot be entirely the existence of different hyaluronidases in different sources (see later).

b. *The viscosity reducing method.* Advantages of this method are its accuracy and its simple kinetics. It has the disadvantage of being cumbersome and not easily carried out in serial experiments. The first systematic use of this method was published by Madinaveitia and Quibell (41) with a crude extract of acetone precipitated vitreous humor as substrate. The time required to reach half

viscosity was found to be inversely proportional to the concentration of enzyme. Details as to substrate and sodium chloride concentrations, pH and temperature vary somewhat in different laboratories. The procedure used in this institute is as follows: 5 cc of a 0.3 per cent solution of pure sodium hyaluronate in 0.1 mol acetate buffer of pH 6.0 containing 0.15 mol sodium chloride are warmed to 37° for about 5 minutes. One cc. of enzyme dissolved in the above buffer mixture is added, and 5 cc. of the mixture are immediately transferred to an Ostwald viscosimeter in a constant temperature bath of 37.0°. The viscosities are determined repeatedly, until less than half viscosity of the mixture is reached. One unit is defined as the amount of enzyme required to reach half viscosity in 30 minutes. The viscosity of the substrate mixture in the control is about 4.0 relative to the solvent. The viscosity of the substrate remains constant for at least 2 weeks at 4° without preservative.

c. The turbidimetric method. This method, described first by Kass and Seastone (34), is based on the observation that pure hyaluronate at pH 4.2 gives a fairly stable colloidal suspension with dilute serum, whereas depolymerized hyaluronate remains clear. The method is reproducible to about 10 per cent, requires little substrate and is readily applied in large series. In a modification developed in this laboratory (21) the time of incubation is kept constant; in the original procedure the time is varied, the enzyme concentration being constant. In this modification the reaction is carried out in the presence of sodium chloride. Dilution of the enzyme in 1 cc. volumes of 0.1 M acetate buffer of pH 6.0 is warmed to 37° for 5 minutes. To each tube 1 cc. of the substrate is added, containing 4 mgm. per cc. of sodium hyaluronate in M/10 acetate of pH 6.0 and 0.3 mol sodium chloride. The mixtures are incubated for 30 minutes at 37°, immersed in a 60° water bath for 10 minutes to inactivate the enzyme, and 1 cc. aliquots are pipetted into test tubes containing 3.0 cc. of 0.5 M acetate of pH 4.2 and 1 cc. of acidified horse serum and mixed. The serum solution is prepared by 10 fold dilution of the sterile normal horse serum (containing no preservative) with 0.5 mol acetate buffer of pH 4.2 and acidifying to pH 3.1 with 4 N hydrochloric acid. The turbidities are read after 30 minutes at room temperature in a Coleman spectrophotometer using a wavelength of 580 mμ. One unit is defined as the amount of enzyme which reduces the turbidity given by 0.2 mgm. of hyaluronate to that given by 0.1 mgm. The values are read from a standard curve. The latter has been checked with 10 different preparations of hyaluronate from different sources. All points fall on the same S shaped curve. In the region between 0.05 and 0.15 mgm. the curve is almost a straight line.

The ratio of the units determined viscosimetrically to those determined turbidimetrically with 4 different hyaluronidase preparations varied only from 1.2 to 1.4. In this comparison the conditions of the two tests were identical with the exception of the substrate concentration.

3. *Chemical methods.* Chemically hyaluronidase activity has been determined by measuring the increase in reducing sugar, or by the increase in liberated acetylglucosamine. In both methods pure hyaluronate of known hexosamine and uronic acid content should be used. The main disadvantage of the reducto-

metric method is its complicated kinetics, obviously the resultant of the action of different enzymes, which simultaneously split glucosidic linkages (see later). In this laboratory the method has been used extensively in the identification of hyaluronate from different sources. Either the Hagedorn-Jensen or the ceric sulfate method in the modification of Miller and Van Slyke was used and the results calculated as per cent of total reducing sugar expressed as equivalents of glucose. The substrate concentration usually was 0.5 per cent.

The increase in acetylhexosamine (68) has been used by some investigators (9) using synthetic acetylglucosamine as standard. It was pointed out that this method with the pneumococcal hyaluronidase indicated freeing of acetylhexosamine in excess of 6 times of the total weight of polysaccharide present (57). A similar result was reported by Hahn for testicular and Cl. perfringens hyaluronidase (19). Whether the measured rate of the color reaction of the enzymatic hydrolysis over that of synthetic N-acetylglucosamine is due to a structural difference in the acetylglucosamine or due to the linkage to the uronic acid in the hydrolysis product has not been decided.

In a recent paper Humphrey (31) confirmed the discrepancy between reducing and acetylhexosamine values on hydrolysis of hyaluronate with enzymes of bull testis, Cl. Welchii, and two strains of streptococci. The excess of the acetylhexosamine varied with different enzyme preparations and was not as high as found in this laboratory with pneumococcal enzyme. He further reported that the enzymic hydrolysates gave a direct colour in acid solution with Ehrlich's reagent without preliminary treatment with alkali, which is necessary to form an oxazoline derivative from acetylglucosamine. The author discusses whether oxazoline rings are preformed in the hyaluronate molecule. The alternative that enzymatically formed acetylglucosamine is labile and easily condenses to a heterocyclic compound seems more probable. The hydrolysis of chondroitin sulfate by testicular enzyme showed no excess of acetylhexosamine over the reducing values (32).

The method obviously cannot be used for the determination of hyaluronidase activity.

C. *The Mechanism of Hyaluronate Degradation by Hyaluronidases.* The dual nature of the two glucosidic linkages in hyaluronic acid, one belonging to the N-acetylglucosamine, the other to the glucuronic acid moiety, suggests that the depolymerization and the hydrolysis into monosaccharides require two enzymes. A comparison of hyaluronidases of different origin, measured viscosimetrically and reductometrically, indicated that hyaluronidases were mixtures of at least two enzymes, one attacking the long chain molecules, the other hydrolysing the aldobionic acid units formed (57). This conclusion was based on the observation that pneumococcal hyaluronidase hydrolysed the substrate almost to 100 per cent of the theoretical amount whereas testicular hyaluronidase, which showed a much faster rate in the viscosimetric tests than the bacterial enzyme, hydrolyzed the substrate to only approximately 50 per cent. The testicular enzyme on prolonged hydrolysis exceeded the 50 per cent reduction, but the total reduction was considerably short of 100 per cent. The addition of pneumococcal

hyaluronidase to the non-hydrolysed residue brought about complete hydrolysis, while addition of fresh testicular enzyme had a negligible effect.

A still greater discrepancy between reductometric and viscosimetric activity was found with leech enzyme (60). One preparation as a flavianate reached a maximum hydrolysis, measured reductometrically of 40 per cent of the theory. Yet the same enzyme preparation turbidimetrically assayed 330 units, that is, it was equal to the best testicular preparations we prepared. This preparation showed an interesting difference from other hyaluronidases in the turbidity test, being uninfluenced by the absence of sodium chloride. Hahn (19) working with a purified enzyme prepared from aqueous extracts of the heads of leeches reported a maximal hydrolysis of only 26 per cent of the theory. Other preparations of leech enzyme in this laboratory analysed reductometrically as high as 70 per cent of the theory. It seems quite obvious that in purification one activity is increased at the cost of the other. Another difference between two hyaluronidases, one from bull testes, the other from streptococci, has been reported by Rogers (78). This author investigated the stimulation of hyaluronidase production by streptococci on addition of hyaluronate to a simplified culture medium. If instead of hyaluronate a digest of hyaluronate obtained with streptococcal hyaluronidase, was added to the culture medium, no stimulation of hyaluronidase production took place. If, however, a digest of hyaluronate obtained with testicular hyaluronidase was added, the stimulation of hyaluronidase production was almost as great as with native hyaluronate. Furthermore, the diffusible fraction of the latter hydrolysate was inactive, while the activity remained in the non-diffusible fraction (unfortunately the hyaluronate in these experiments was rather impure). Recently, similar observations were reported on the hyaluronidase production of Cl. Welchii (79).

Other evidence for the existence of several enzymes was brought forward by Hahn (20) who claims to have separated two enzymes from testicular extracts. One enzyme, called mucopolysaccharidase (abbreviated M.P.) hydrolyses to the aldobionic acid stage, the other muco-oligosaccharidase, (abbreviated M.O.) hydrolyses the disaccharide to monosaccharides. Since the M.P. liberates acetylglucosamine during hydrolysis, the free aldehyde group must belong to the hexosamine moiety, therefore the M.O. was called a glucuronidase. The two enzymes were fractionated by $(NH_4)_2SO_4$ and lead acetate, the M.O. activity precipitating in the first fractions. M.P. activity was measured by a viscosimetric method, and M.O. activity by a reductometric method. The substrate of the latter was hyaluronate previously digested for 4 days with M.P. followed by dialysis, the concentrated non-diffusible residue being the substrate. There was no linear relation between the percentage of hydrolysis and the enzyme concentration. The total reducing values must have been quite small, since the substrate concentration was very low, only 0.5 mgm. glucose equivalent per cc., and a turnover of only 10 per cent was measured.

To this reviewer it seems highly desirable to test the presence of the M.O. with a well defined substrate. One would expect the disaccharide formed by the 4 day hydrolysis to be diffusible. There also remains the question of whether the M.O.

can or cannot attack the glucuronidic linkage in the native hyaluronate. The hydrolysis of the linkage in the native chain presumably would also cause a decrease in viscosity, while the end groups with the two enzymes ought to be different.

One distinct enzyme, β-acetylglucosaminidase, present in crude testicular and in crotalus hyaluronidase, as well as in sources showing no hyaluronidase activity, does not seem to be involved in hyaluronidase activity (13). This enzyme was found by Helferich and Iloff (24) in emulsin of sweet almonds. The substrate used was β-phenylacetylglucosamine. The enzyme was separated from testicular hyaluronidase with $(NH_4)_2SO_4$, lead acetate or $CuSO_4$ or adsorption on kaolin. Hahn (20) likewise has reported good evidence for the separation of hyaluronidase from β-glucosaminidase activity.

In summary, hyaluronidases of different origin undoubtedly contain different enzymes, but their number and mode of action are not fully known.

D. *Influence of Environmental Factors on Hyaluronidase Activity.* 1. *Effect of pH.* The effect of pH on the rate of hyaluronidase activity varies with the source of the enzyme, the salt concentration and the test method. In the reductometric procedure testicular hyaluronidase has a double optimum, one at about pH 4.5, the other at about pH 5.7 which has been interpreted as another indication for the presence of two distinct enzymes (57). The pH optimum of pneumococcus and Cl. Welchii hyaluronidase was found in the reductometric procedure at 5.8 (62). By a viscosimetric method, McClean (48) found a marked dependency on the salt concentration of the activity at different pH levels. In M/60 buffer the optimum of testicular hyaluronidase was at pH 6.8, while in M/6 buffer, the optimum shifted to pH 5.0. A similar behaviour was found with Cl. Welchii enzyme. Moreover, at the pH optimum the activity of the enzyme was much greater in the more dilute buffer. The marked difference found may have been partly due to differences in chloride ion concentrations (see later), especially with hyaluronate of umbilical cord. With the turbidimetric method, the pH optimum of testicular hyaluronidase in M/10 acetate in presence of 0.15 M sodium chloride was found to be 6.0 (21).

2. *Effect of salts.* Robertson et al. (76) reported that dialyzed Cl. perfringens hyaluronidase had no effect on a dialyzed "synovial mucin." Addition of phosphate or other salts in increasing concentrations (up to 0.1 M) resulted in increasing activity. Madinaveitia and Quibell (42) reported a marked influence of salts, especially sodium chloride, on the activity of testicular hyaluronidase, as determined viscosimetrically. The optimum was between 0.07 and 0.17 mol sodium chloride.

In this laboratory the influence of salts, especially sodium chloride, was found to be much more marked with the turbidimetric and viscosimetric methods than with the reductometric. The same behaviour had been shown with lysozyme and its substrate (59). The influence of sodium chloride on the rate of depolymerization depended further on the source of the substrate. This is illustrated in the following table in which the same enzyme preparation, highly purified testicular hyaluronidase, has been tested.

It can be seen from this table that the apparent activity of a sample varied from 100 to 167 units per mgm. The wide variation in apparent activity in the absence of NaCl should be noted. By the quantitative turbidimetric test the different hyaluronates precipitated an equal amount of serum protein. Analytically, they were identical within the limits of the errors of the methods. Quantitative determinations in 100 mgm. of the tumor preparations after ashing with HNO_3 showed only a trace of chloride. The chloride effect and the differences between the hyaluronate preparations seem to be due to the presence of competitive inhibitors, as described in the next chapter. It is evident from these experiments that the purity and origin of the substrates greatly influence the titres of enzyme preparations.

3. *Inhibitors of hyaluronidases.* McClean (47) reported the inhibitory action of heparin and chondroitin sulfate on the in vitro decapsulation of streptococci by testicular hyaluronidase. Three-tenths per cent of the pure NH_4 salt of heparin suppressed the decapsulation of an enzyme concentration equivalent to one viscosity reducing unit. From 0.001 to 0.01 per cent of the NH_4 salt further inhibited the action of one unit of hyaluronidase on 0.1 per cent hyaluronate. Hyaluronate partly depolymerized by precipitation with acetic acid likewise

TABLE 1

SOURCE OF HYALURONATE	NO NaCl; UNITS/MGM.	0.15 M NaCl; UNITS/MGM.
Umbilical cord	5	100
Tumor	67	167
Synovial fluid	67	100
Strep. C	25	125

inhibited the decapsulation, while hyaluronate depolymerized enzymatically was without inhibitory effect. Gastric mucin also inhibited the decapsulation, but Shiga-Kruse polysaccharide and a blood group A hapten had no effect. It should be noted that all inhibitors are or contain acid polysaccharides; the two neutral polysaccharides tested were without action.

In this laboratory the inhibiting activity of heparin on the hydrolysis of hyaluronate was tested and results are shown in table 2. Heparin desulfurated with oxalic acid-barium oxalate had no effect. The inhibition with chondroitin sulfate was considerably less than with heparin, the ratio between the two being approximately 1:100.

The inhibitory action of normal human and rabbit serum on pneumococci, Cl. Welchii and streptococcus hyaluronidase seems to have been reported first by this laboratory (26). The reductometrically determined activity of the first two was inhibited 25 to 50 per cent by normal sera, while with streptococcus hyaluronidase the inhibition was smaller. McClean (47) reported the inhibition of guinea pig, rabbit, sheep, horse, mouse and human serum on hyaluronidase prepared from bull, rabbit and mouse testes, from streptococcus, Cl. Welchii, and from viper and scorpion venom. The inhibitory activity of the sera from

different species against any one enzyme showed considerable variation. The inhibitory action of the sera was associated with the pseudoglobulin fraction. McClean suggested that the inhibition was due to some polysaccharide fraction of the serum.

Meyer et al. (62) found the hydrolysis of free hyaluronate twice as fast as that of an equimolar concentration of a protein salt of the acid, prepared from synovial fluid. In experiments of Kass and Seastone (34) the reduction of the turbidity of a preformed horse serum-polysaccharide precipitate required 10 times the enzyme concentration than that of the polysaccharide alone. More recently Leonard and Kurzrok (36) reported that normal sera inhibit the liberation of tubal ova by testicular hyaluronidase (see later).

The inhibitory action of serum on hyaluronidase of animal and bacterial origin recently was the object of three papers (18). A complete discussion of all the experimental evidence presented and the conclusions drawn from them seems

TABLE 2

	UNITS/MGM.	% INACTIVATION
Hyaluronidase + Hyaluronic acid	83	
Hyaluronidase + Hyaluronic acid + NaCl	200	
Hyaluronidase + Hyaluronic acid + 0.040 mgm. heparin	3	96
Hyaluronidase + Hyaluronic acid + 0.040 mgm. heparin + NaCl	200	0
Hyaluronidase + Hyaluronic acid + 0.004 mgm. heparin	12	85
Hyaluronidase + Hyaluronic acid + 0.0004 mgm. heparin	40	52
Hyaluronidase + Hyaluronic acid + 0.00004 mgm. heparin	83	0

impossible in this review and does not seem warranted. The invasive agent, hyaluronidase, is counteracted by enzymes of the sera of different species, ranging from man to carp, which were said to destroy hyaluronidase. Crude bacterial and some animal hyaluronidases in turn contain other invasive enzymes, which counteract the defensive enzymes of the sera, which in turn are again counteracted by another serum enzyme and so forth.

Experimentally, hyaluronidase was incubated with serum in a salt buffer mixture and the solution mixed with a very impure hyaluronate of umbilical cord in the presence of phosphate. The hyaluronidase activity was measured viscosimetrically. Phosphate was found by Haas to inhibit the action of serum on hyaluronidase, while other salts, especially borate, promote it. The enzymatic nature of the inhibiting agent, in serum was concluded from the temperature sensitivity, the dependence on pH and the inactivation by heating. This evidence appears inconclusive. The activity of sera among comparable data in different tables is very variable. The rate of inactivation was reported to depend

considerably on the quality of the polysaccharide (18, p. 78). (A sample of hyaluronate prepared according to Haas in this laboratory contained 16 per cent of sodium hyaluronate and over 50 per cent inorganic material.) Furthermore, the inhibition by human sera depends not only on concentration but likewise on purity of the hyaluronidase (21). That is, the protein impurities present in hyaluronidase likewise react with the serum. The inactivation by sera is not only prevented by phosphate, but at least in part reversed on further incubation. The inhibitory action of serum furthermore is so dependent on the milieu that it seems very difficult to draw conclusions from experiments in which the complex systems are further complicated by adding crude bacterial filtrates or snake venoms. To this reviewer the data presented do not seem essentially different from the inhibition by heparin and other substances, except that the inhibitors in serum are more thermolabile. The great dependency on milieu conditions is interpreted by this reviewer as being due to competitive reactions between various proteins among themselves and for the acid substrate. That hyaluronidase can be active in the presence of serum proteins is shown not only by its spreading reaction in the skin, but its action in pathological human joint fluid and other fluids and its in vivo activity on sedimentation rate of blood when injected intravenously.

4. *Antisera to hyaluronidases.* The inhibition of the spreading reaction by specific antisera has been discussed by Duran-Reynals (12). Various papers published since then support the previous conclusions that antisera to hyaluronidases suppress the in vivo and in vitro activities of hyaluronidases, but that they are strictly specific for the source of the enzyme. Thus McClean (48) finds no crossing over with antisera against Cl. Welchii to Cl. septicum. Streptococcal enzymes were found to be group, but not type specific. An antiserum against crude bovine testicular hyaluronidase neutralized bovine, but not mouse testis hyaluronidase. Highly purified testicular hyaluronidase, however, has never been obtained in antigenic form (49).

E. *Bacterial Hyaluronidases.* Increase in hyaluronidase production by the addition of hyaluronate to the medium was first reported by McClean and Hale (49) for Cl. Welchii. The stimulating effect of added hyaluronate was studied systematically by Rogers (77, 78). In groups A and C hemolytic streptococci and in two strains of Cl. Welchii enzyme production was proportional to the concentration of hyaluronate added and rose from a titre of 1:50 M.C.P. units to as high as 1:100000 units, when the pH was maintained at neutrality by 8.5 per cent sodium glycerophosphate. One of two strains of staph. aureus produced a titre of 1:3200 units, which was not increased by hyaluronate, the other had a low titre with or without added hyaluronate. The titre was not raised either in a strain of Cl. septicum (77, 78). In staph. aureus the hyaluronidase production was raised by addition of a papain digest of peptone to the medium, while in Cl. septicum it could be increased by some specially prepared peptone (79).

A systematic study of hyaluronidase production in relation to serological groups and types, to capsule formation and to the sources of the strains in hemoly-

tic streptococci was published by Crowley (11). A total of 376 strains were tested, 308 of which belonged to group A. Of the latter, 64 strains, all belonging to types 4 and 22, showed hyaluronidase activity. Seventy-two and 96 per cent respectively of all types 4 or 22 examined were hyaluronidase producers. The titres varied from 50 to 1000 M.C.P. units/cc. Thirty-five out of 55 group C strains and all of 13 examined of group G strains of a variety of types showed hyaluronidase activity. All hyaluronidase positive strains were non-capsulated, but many of non-capsulated strains were hyaluronidase negative. Little correlation was found between hyaluronidase production and virulence. Of streptococcus strains isolated from 127 cases of scarlet fever, 27.6 per cent; of 136 cases of sore throat, 36.8 per cent showed hyaluronidase activity. Of the latter half of the strains belonged to groups C and G. Twenty and four-tenths per cent of hyaluronidase positive strains were isolated from wounds, burns or impetigo contagiosa and 23 per cent from healthy throats. Of 6 cases of puerperal fever, 3 were hyaluronidase producers, among them a group C strain isolated from the blood of a fatal case. However, in another case where the organism was isolated from the blood stream, it was capsulated and negative for hyaluronidase. In some cases highly contagious strains were isolated from contacts in different individuals. The organisms were capsulated and none produced hyaluronidase. The author concludes that there was no evidence for correlation of hyaluronidase production of streptococci with any particular type of infection, nor with virulence of streptococci for man.

Hyaluronidase production of streptococci as reported by Crowley merits some discussion, especially in regard to negative findings in non-encapsulated strains. It was reported from this laboratory (57) that streptococcal hyaluronidase, present in the medium as well as in purified preparations, showed a rapidly diminishing activity as compared to pneumococcal hyaluronidase. Furthermore, the same strains of both group A and C organisms varied greatly in hyaluronidase production from completely negative to high concentrations, although grown under apparently identical conditions. In animals the culture medium and purified samples gave a pronounced spreading reaction regardless of the in vitro titre of hyaluronidase activity. It was suggested that the enzyme may be inactivated, the inactivation being reversible in vivo, while no reversibility could be demonstrated in vitro. Hale (22) confirmed the anomalous behaviour of streptococcal hyaluronidase in the viscosimetric tests but only when carried out at pH 4.6. Furthermore, exposure of the enzymes of groups A and C streptococci to pH 4,6 before mixing with the substrate resulted in complete inactivation of the enzyme. At neutrality the enzyme showed a normal fall in viscosity proportional to the enzyme concentration. In our experiments the substrate was the sterile pleura fluid of a patient with mesenthelioma. The reaction was carried out at a pH of about 7.0. The reductometric tests were carried out at pH 5.8 with pure hyaluronic acid as the substrate. Inactivation of the enzymes was apparent with both methods. Whether or not the inactivation is enzymatic has not been determined.

In view of these experiences with streptococci it may be questioned whether

failure to demonstrate hyaluronidase is synonymous with absence of the enzyme. A renewed study of hyaluronidase in streptococci seems indicated, not only in regard to hyaluronidase production, but also in regard to spreading activity, which in our experience is not confined to types 4 and 22 of group A organisms.

The correlation between capsule formation and hyaluronic acid production has been studied by Seastone (82) in a group C streptococcus. The evidence there given for the rôle of the hyaluronate capsule in the virulence of the infecting organism has been corroborated by Hirst (25), who protected mice by the intraperitoneal injection of leech extracts against fatal infection with a strain of group C streptococcus, while against a group A strain only feeble protection resulted. Blundell (5) obtained a greater mean survival time in mice infected with a group A organism, when treated with testicular hyaluronidase than without treatment. McClean (47) reported failure to protect either against groups A or C organisms with testicular hyaluronidase. Kass and Seastone (34) protected mice against 10 to 100 M.L.D. of groups A and C organisms by repeated injections of testicular hyaluronidase. No protection resulted from injection of inactivated hyaluronidase. The specificity for streptococcus was demonstrated by the failure to protect against the fatal infection with a type I pneumococcus. The failure of other authors to obtain this effect was explained by the time lag between injections of the enzyme, which allowed the organisms to regenerate their capsules. Kass and Seastone further studied the in vitro effect of hyaluronidase on streptococci. Phagocytosis of group A organisms by human leucocytes and the killing power of whole blood was greatly increased by incubation of the organisms with hyaluronidase. In controls with pneumococci no change resulted from incubation with hyaluronidase.

In *pneumococci* no correlation between the amount of hyaluronidase produced and the clinical virulence, or between enzyme production and the type of organism, was found (30). Type I organisms rarely produced the enzyme.

In *staphylococcus aureus* no correlation between enzyme production and virulence was reported by Boe (6). However, Schwabacher et al. (81) found among 654 coagulase positive strains of staphylococci 86.7 per cent producing both α-lysin and hyaluronidase, 6.9 per cent hyaluronidase positive and α-lysin negative, 4.4 per cent hyaluronidase negative and α-lysin positive and 2 per cent negative for both. None of the 160 coagulase negative strains produced either α-hemolysin or hyaluronidase. The author concludes that clinical virulence is associated fairly clearly with α-hemolysin production and to some extent with hyaluronidase production. What part hyaluronidase plays in determining the virulence of a strain of Staphylococcus was not clear.

In Cl. Welchii, McClean, Rogers and Williams (52) found 12 out of 32 strains produced hyaluronidase. Of these 12 strains 11 produced toxin and 10 were derived from clinical cases of gas gangrene. In Cl. septicum 20 out of 20 strains in vitro produced hyaluronidase. Of these 4 were derived from clinical cases of gas gangrene, 9 were stock laboratory strains and 7 were contaminants of "healthy" wounds. Seven out of 15 strains of Cl. oedematiens produced a low

titre of hyaluronidase. Six of these were stock laboratory strains and one was derived from a toxemia in sheep. McClean et al. (52) proposed the determination of hyaluronidase and lecithinase for early diagnosis of gas gangrene in wound infections. Guinea pigs were infected intramuscularly with organisms of the gas gangrene group and the presence of the enzymes was determined in the muscle extracts and edema fluid. With Cl. Welchii as infective agent the muscle contained 2 M.C.P. units 2 hours after infection. With Cl. septicum the infection developed more slowly than with Cl. Welchii. Edema fluid was not obtained before 12 hours after infection, when it as well as the muscle did contain hyaluronidase in low concentrations. With Cl. oedematiens the infection proceeded still more slowly than with Cl. septicum. In some instances 12 hours after infection hyaluronidase was detected in the edema fluid, but since many strains did not produce hyaluronidase in vitro, this organism was the least satisfactory from the point of view of detection of enzymes in the body fluids. The authors further proposed to utilize specific antisera for the differential diagnosis of the infecting agents in combination with the enzyme determinations, as in positive tests only the specific antisera will inhibit the enzyme of the infecting agent (50).

Clinically the proposal of McClean et al. was tested by MacLennan (39) in 39 cases, among them 12 cases of clinical gas gangrene, the others from various infections or from non-infected wounds. Only in one case, infected with a streptococcus pyogenes, was a positive M.C.P. test obtained.

McClean and Rogers (51) ascribed the failure of MacLennan at least in part to the treatment of the clinical cases with antisera. In guinea pigs the administration of antisera inhibited and sometimes suppressed the appearance of hyaluronidase and lecithinase. They further tested whether the size of the infecting dose explained the difference between the animal experiments and clinical cases. A minimal infecting dose of organisms did not diminish the titre in the infected tissues of the guinea pig. They further studied a combination of infecting agents. Cl. histolyticum, which produced no hyaluronidase, caused death in combination with a streptococcus earlier than Cl. histolyticum alone. The strain of streptococcus used did not produce illness in the guinea pig either in presence or in absence of added hyaluronidase. A combination of Cl. histolyticum with the streptococcus and added hyaluronidase caused death even earlier than in the previous experiments.

MacLennan and associates (40) maintained in a later article that the determination of hyaluronidase and lecithinase was unsatisfactory as a diagnostic procedure.

F. *Substrates of Hyaluronidase.* The difference in the apparent activity of testicular hyaluronidase on hyaluronate of different sources has been discussed above. Highly purified testicular hyaluronidase has been stated to hydrolyze beside hyaluronate only two other mucopolysaccharides, one a monosulfuric acid ester of hyaluronic acid obtained from cornea (54), the other the chondroitin sulfate of hyaline cartilage (57) (43). The hydrolysis of chondroitin sulfate is

of considerable interest, since compounds of the general composition of this acid occur beside hyaluronate, in mesodermal tissue, like umbilical cord (64) and skin (56) in concentrations about equal to that of hyaluronate. The chondroitin sulfate of skin differed in rotation from that of cartilage (56). The two acids were found, in collaboration with Dr. Z. Dische, to differ further in their resistence to alkali and considerably in the time-intensity curve of their reaction with carbazole. Moreover, chondroitin sulfate of skin, and to a lesser degree of umbilical cord, in contrast to that of cartilage were resistant to hydrolysis with testicular hyaluronidase.

Is chondroitin sulfate of cartilage a substrate of hyaluronidase, or is it hydrolysed by another enzyme copresent in the testicular preparations? The evidence points to the latter explanation. Chondroitin sulfate is not attacked by pneumococcal or streptococcal or leech hyaluronidases. While it might be argued that the bacterial enzymes are to a lesser degree true mucopolysaccharidases, this cannot be claimed for the leech enzyme. Furthermore, purified testicular hyaluronidase after precipitation by acetone lost most of its chondroitin sulfate hydrolysing power, while the hydrolysis of hyaluronate was hardly impaired. However, it seems remarkable that hyaluronidase activity towards hyaluronate runs practically parallel with the activity towards chondroitin sulfate, whether the fractionation is carried out with metal salts or with adsorption. The enzyme hydrolyses chondroitin sulfate with the production of a disaccharide without hydrolysing the sulfate linkage. Humphrey in a recent paper (32) found no hydrolysis of chondroitin sulfate with streptococcal enzyme and a negligible effect with an enzyme prepared from Cl. Welchii. Crude and a highly purified testicular enzyme however at pH 4.8 and 6.0 liberated reducing sugars with chondroitin sulfate at a rate comparable to hyaluronate. Some bacterial extracts have been reported to hydrolyse chondroitin sulfate by splitting off inorganic sulfate (69).

III. HYALURONIC ACID AND HYALURONIDASE IN ANIMAL PHYSIOLOGY AND PATHOLOGY. A. *Ocular Fluids.* Hyaluronic acid has been isolated from vitreous humor of cattle, swine, and sheep and from the aqueous humor of cattle. Its concentration in the normal fluids has been determined by analysis of the hexosamine content. This procedure involves only a small error, since the protein, being very low in these fluids, contributes very little to the total hexosamine. The concentration of hyaluronic acid found by isolation was in fair agreement with that calculated from the hexosamine values. The polysaccharide content of vitreous humor varies considerably with the species, from a low of about 9 mgm. per cent in the cat to a high of about 48 mgm. per cent in cattle eyes, while the concentrations in aqueous humor showed little variation (67).

The origin of the polysaccharide in the ocular fluids is not known with certainty. While the permanent structures of the vitreous body seem to be of retinal origin (44), the fluid part probably originates mainly in the ciliary epithelium which (in this country at least) is considered a secretory epithelium. The presence of hyaluronate in the fluids and its absence from serum have been cited as evidence for its origin from a secretion rather than a dialysate (65). Hyaluronate on injection into the anterior chamber of rabbits disappears rather

rapidly. After paracentesis of the anterior chamber the acid reappears (as judged by analysis of the hexosamine) after the return of the protein content to normal. It was concluded that the acid was constantly produced and removed, presumably after enzymatic hydrolysis. Hyaluronidase has been reported in extracts of the ciliary body and iris (67) though Chain and Duthie (9) denied its occurrence in these tissues and suggested bacterial contamination as a source of the enzyme. The presence of hyaluronidase has been definitely established in pooled aqueous humor obtained by aspiration of the eyes of freshly killed cattle. The aqueous humor was lyophilised and the residue dissolved in 1/10 of the original volume. Four units (turbidimetric) of hyaluronidase were found per cc. of the concentrate, corresponding to 0.36 unit per cc. of the native fluid (21). It is of considerable interest that the hyaluronate present in the same sample was found to be almost completely depolymerized. While the vitreous humor of the same eyes had a hyaluronate concentration (turbidimetric) of 0.75 mgm. per cc., the lyophilized aqueous humor showed only 0.00015 mgm. per cc. By hexosamine estimation the hyaluronate concentration of the vitreous humor ranged from 0.32 to 0.52 mgm. per gram (the lower figures are probably explained by the difference in specific gravity and evaporation of water). In aqueous humor the hyaluronate concentration was about 0.03 mgm. per gram, a value in fair agreement with the concentration found by isolation of an impure fraction (0.046 mgm. per gram). Since the concentration by the turbidimetric method amounted only to 0.0015 mgm. per cc., we may conclude that the hyaluronate in aqueous humor is depolymerized to about 95 per cent of the total. As this depolymerization presumably occurred in vivo, we assume that hyaluronate is after enzymatic depolymerization constantly removed through the normal exit channels of the eye.

What part hyaluronate plays in the physiology and pathology of vitreous humor is unknown. It probably takes part in the maintenance of the turgor of the vitreous body. The effect of intravitreal injections of purified testicular hyaluronidase in rabbits is being studied by Dr. L. von Sallmann. Injections of about 100 turbidimetric units appear to liquefy the vitreous humor in vivo. The cause of simple glaucoma may well be explained by inhibition of hyaluronidase in the eye.

B. *Synovial Fluid.* Bauer et al. (1) have reviewed the physiology of synovial fluid. The view expressed in their paper that synovial fluid contains a glycoprotein instead of a dissociated mucopolysaccharide seems no longer tenable (see p. 337). Furthermore, the concept of the origin of synovial fluid as a dialysate to which the "mucin" is added by the passage of the fluid through the connective tissue does not seem probable. If such a mechanism existed then pleura and peritoneal fluid and lymph should likewise contain the "mucin" which is not demonstrably the case. From the appearance of a viscous fluid in tissue culture of synovial tissue (86) it may be concluded that hyaluronate is a secretory product of some cells of the synovial lining. This seems to be borne out by the isolation of hyaluronic acid from a synovioma, not only at the original site of the tumor, but in metastases in the liver (60).

The concentration of hyaluronic acid in normal human synovial fluid has not

been determined by isolation (see below). In cattle synovial fluid obtained by aspiration of astragalo-tibial joints the concentration by isolation was found between 20 to 25 mgm. per cent (66). From knees of patients with rheumatoid arthritis the acid was isolated in 3 different samples in concentrations of 60, 132 and 206 mgm. per cent. However, it may be questioned whether cattle synovial fluid as collected, is normal and comparable to the fluid in human joints. Its viscosity is low compared to that of normal human fluid taken at autopsy or from living patients with no pathology of the joints (72). By extrapolation of the dilution curve of human synovial fluid the relative viscosity of the latter was estimated as greater than 180, whereas that of the cattle fluid was less than 10. The volume of the fluid of cattle taken immediately after slaughter is quite large, sometimes over 50 cc. It seems possible that due to long standing and trauma of the animals before killing the cattle fluid is actually a mixture of synovial and edema fluid.

The hyaluronic acid and protein contents and the viscosities of about 30 pathological fluids have been determined (74). The viscosity was variable and never higher than that of normal knee joints. The protein content was over 3 per cent, that of normal human and cattle fluid below 3 per cent. The viscosity practically was all due to hyaluronate, since on incubation with enzyme the relative viscosity fell to slightly above 1.0, corresponding to the viscosity of the remaining protein solution (66).

By the turbidimetric method the hyaluronic acid content varied from 80 to 270 mgm. per cent. No direct proportionality between viscosity and hyaluronate concentration was found. In normal human and in cattle synovial fluid the hyaluronic acid concentration could not be determined turbidimetrically by the above method. In contrast to the pathological fluids the normal fluids in the turbidimetric procedure are precipitated as a coherent fibrous clot while the pathological fluids with few exceptions precipitated in the form of a stable turbidity, as do vitreous humor and the tumor fluids. On incubation of the normal fluids with 0.01 unit of enzyme, far too little to decrease the apparent hyaluronate concentration, the clot formation of the normal fluids is prevented and hyaluronic acid can be determined turbidimetrically. The amount found varied between 80 and 150 mgm. per cent as compared to 80 to 270 mgm. per cent in pathological fluids. That is, both the concentration and especially the total amount of the fluid in pathological joints is larger than that found in normal joints. Hyaluronidase in contrast to normal ocular fluid could not be demonstrated in synovial fluid, although the absence of fibre formation may suggest its presence in low concentrations in pathological joint fluids.

C. *Hyaluronic Acid in Tissues.* The presence of hyaluronate in tissues when isolation is not possible, can be made probable enzymatically by the use of a variety of bacterial and animal enzymes. Judging from the failure of isolation experiments and the absence of any effect on capillary permeability by purified hyaluronidase, hyaluronic acid is not present in capillary cement. The presence of hyaluronic acid in all connective tissues has not been established. Positive spreading reactions in stomach and intestinal walls, in the uterus, in striated

muscle, fasciae and tendons with crude testicular extracts have been reported (12, 14). It remains to be determined whether the spreading is due to depolymerization of hyaluronate or chondroitin sulfate. In calves' tendon the polysaccharide is a sulfate ester and is hydrolyzed by testicular hyaluronidase at a fast rate (60). Only in skin and in umbilical cord do larger quantities of hyaluronate occur. It seems remarkable that in both tissues chondroitin sulfate is present in a concentration roughly equal to that of hyaluronate. Both substances have to be regarded as constituting essential components of the interfibrillar ground or cement substances in these tissues. It seems of great importance to gain more information about the nature of such cement substances especially for the understanding of the mechanism of diseases of the mesenchymal tissues as well as of wound healing and the problem of ageing.

Beside hyaluronate and chondroitin sulfate the mucopolysaccharide isolated from amyloid tissue may likewise be such a cement substance. This substance is a monosulfuric acid ester, containing acetylhexosamine and a uronic acid in equimolar proportion. The hexosamine has been isolated and characterized as d-glucosamine (60). The behaviour of the mucopolysaccharide towards hyaluronidases shows that it is not derived from hyaluronic acid. From its enzymatic behaviour and its reactions with alkali and with carbazole it appears to be closely related to heparin. It may well be possible that this substance accumulates in excessive amounts in certain pathological conditions, while in small concentrations it may be a normal component of some mesenchymal tissues.

Histologically the presence of hyaluronic acid has not been demonstrated, since no staining method was known. Hale (23) recently proposed a histochemical test for hyaluronic acid. After a specific fixation of the tissue, slices are treated with iron hydroxide. The iron combines with the acid polysaccharides. The bound iron is stained as Prussian blue. For the differentiation between hyaluronate and other polysaccharides incubation of the tissue slices with streptococcal hyaluronidase is recommended, since this enzyme does not attack chondroitin sulfate. No experimental results with this method have been reported.

Highly polymerized hyaluronate in a concentration of about 1 per cent shows in smears typical metachromatic staining with toluidine blue, while lower concentrations failed to show the effect. The metachromasia is prevented on incubation with hyaluronidase (unpublished experiments). The concentrations present in tissues are probably never high enough to contribute to the metachromatic staining. Therefore, the metachromasia of tissues, done under appropriate conditions, probably does indicate sulfate containing compounds (37) in connective tissue probably largely chondroitin sulfate. The viscous material demonstrated by Bensley (2) in regenerating and young tissue and by Maximow (45) in tissue culture, possibly indicates hyaluronate. The rôle of calcium and ascorbic acid in the cement substances of plants and animals has been discussed by Reid (74A).

Robb Smith (75) has studied histologically in vitro the effect of testicular and streptococcal extracts on the muscle of guinea pigs. Reticulin or collagen

were reported not to be affected, but the reticulin membranes became separated from the muscle fibres. The author suggests that there may be a layer of mucopolysaccharide between the reticulin membrane and the sarcolemma. The changes were absent in heated extracts and were prevented by streptococcus antisera when streptococcal hyaluronidase was employed.

A tentative picture of the development of cement substances in mesodermal tissue combining the admittedly scanty data may be presented as follows: The young fibroblast secretes into the surrounding tissue spaces hyaluronic acid, a precursor of collagen, and a chondroitin sulfate. By local acidification in the immediate neighborhood of the cells the first fibres are produced by the polysaccharides from the native soluble collagen, which denature into the insoluble fibre, on the surface of which lies a sheet of the polysaccharides. With ageing of the fibres, the polysaccharide layer becomes thinner and the hyaluronate is replaced more and more by chondroitin sulfate. Only in metabolically very active connective tissue like that of skin, hyaluronic acid production continues in appreciable quantities. The rôle of ascorbic acid (87) in the process of fibre formation may be that it actually is a component of chondroitin sulfate, replacing in the chain some of the glucuronic acid molecules.

D. *Hyaluronic Acid and Erythrocyte Sedimentation Rate.* Hyaluronic acid in common with other asymmetrical molecules increases the erythocyte sedimentation rate in vitro or after intravenous injections (61). It was further observed in this laboratory that purified testicular hyaluronidase decreased in vitro the sedimentation rate of blood of patients, especially in rheumatic fever. This action was first thought to be due to the hyaluronidase contained in the enzyme preparations. However, it became apparent that hyaluronidase activity as determined by chemical or physico-chemical methods was not proportional to the action on sedimentation rate. This became still more obvious when it was found that enzyme preparations fractionated by lead acetate no longer acted on sedimentation rate, when lead was removed by Na_2S instead of dialysis, while the hyaluronidase activity was not altered. In further experiments by Dr. C. Ragan (unpublished) the mechanism of the action on erythrocytes was found to be due to spherocyte formation, caused by an enzymatic action of unknown nature on the erythrocyte membrane.

The effect of hyaluronate on erythrocytes seems comparable to the precipitation of large particles, such as hemocyanine, liver particles and a number of viruses by some colloids of high molecular weight (10). The precipitation occurs at neutrality and apparently is not due to polar forces. Depolymerized hyaluronate had no effect. It seems remarkable that hyaluronate of high polymerization had a much larger effect quantitatively than either chondroitin sulfate or heparin (see fig. 1 of Cohen and personal communication). The mechanism of this action does not seem clear, but may be connected with complex coacervation (7) and obviously deserves further study.

E. *Hyaluronidase in the Animal Body.* The rôle of testicular hyaluronidase in testis probably is confined to the dispersion of the cumulus cells in the process

of fertilization (53) (80) (15A). Apparently only mature spermatozoa contain a high concentration of the enzyme. Testes of birds, amphibia and reptiles contain little (12). The mucus of the cervical plug is not acted upon by hyaluronidase (53) (80). The hyaluronidase of human semen has been investigated by several workers (32A) (35A) (86A). Kurzrok et al. by a modified M.C.P. test found a critical value for hyaluronidase of 50 million sperm per cc. Hyaluronidase activity did not run parallel to sperm population. Some apparently normal semen was found to be deficient in hyaluronidase, concomitant with inability to cause fertilization. Mixing of semen and testicular hyaluronidase or application of hyaluronidase in powder form to the cervix was said to have increased considerably the number of successful fertilizations.

No conclusive data are available for the occurrence of hyaluronidase in blood. Intravenously injected enzyme is rapidly eliminated from the circulation.

The demonstration of hyaluronidase in aqueous humor has already been mentioned.

A relatively large concentration of hyaluronidase occurs in skin (57). In press juice of ground rabbit skin very little activity can be demonstrated, but when the material is autolysed in presence of toluol in phosphate buffer of pH 5.0, a relatively large concentration is found in the supernatant solution. Chain and Duthie (9) were unable to find any hyaluronidase in skin. However, the authors likewise failed to obtain a spreading reaction with skin extracts, in contrast to Duran-Reynals' (12) and our experiments. The higher concentration in autolysed material may be due either to the liberation of enzyme bound to cell structures or conversion of inactive into active material. The latter hypothesis seems more likely. Of interest in this connection is the observation of Duran-Reynals that washed glandular tissues of poisonous snakes are almost free of hyaluronidase, whereas it appears with the glandular secretion.

The concentration of potential hyaluronidase in skin may be still higher than that found, since during autolysis considerable destruction of added enzyme occurs.

The occurrence of hyaluronic acid and hyaluronidase in skin may point to a rather rapid turnover of the former. This turnover may be regulated by certain hormones. Luria and Zappasodi (38) found a significant increase in the spreading effect in rabbits treated with luteinizing hormone. Other workers (84) have found a decreased reaction under the influence of follicular hormone. The edema fluid of the sex skin of monkeys in the estrus phase has been reported to be similar to synovial fluid (9). The turgescence of the sex skin of the baboon during the menstrual cycles was studied by Clarke (9A). A considerable part of the water uptake of the skin was apparently bound water since no free edema fluid could be demonstrated. The deturgescence at the end of the estrus cycle supplied the animal with fluid for 11 days. We may assume that this fluid was bound by hyaluronic acid.

Localized hyaluronidase action has been suggested as a possible explanation

for the bullae formation in pemphigus (38A). However, after intradermal injection of hyaluronidase into normal people and into patients suffering from a variety of rheumatic diseases, bullae were never observed.

F. *Clinical Application.* The use of hyaluronidase in artificial insemination has been mentioned above.

On injection of purified testicular hyaluronidase into the knee joints of patients suffering from rheumatoid arthritis the viscosity of the fluid was remarkably reduced. No change was observed in the underlying disease. One week after the injection, the viscosity of the fluid had returned to the original level (73).

In one patient having a mesenthelioma of the pleura and peritoneum purified testicular hyaluronidase of bull or ram was injected intraperitoneally to facilitate removal of a fluid of honey-like consistency (85). Without hyaluronidase injection, evacuation of the fluid was difficult and incomplete. After injection of 16,000 to 80,000 units of hyaluronidase, fluid of low viscosity could be completely removed in a short time. No immediate harmful effects of the injections were apparent. Injections and paracentesis were repeated at various intervals. The tumor finally did not seem to produce as much fluid as originally. On autopsy large tumor masses were found in peritoneum and pleura with little fluid mostly encapsulated.

A connection between hyaluronic acid and hyaluronidase and rheumatic diseases, especially rheumatic fever, has probably been suspected by many workers. In a number of articles Guerra (16) recently reported the inhibiting effect of sodium salicylate on the spreading of India ink, in rabbits injected with crude testicular hyaluronidase. The area of spreading without hyaluronidase was reduced by 20 per cent with 0.07 and by 31 per cent with 0.10 gram per kgm. of sodium salicylate. With hyaluronidase injection the area of spreading was reduced 57 per cent with 0.07 gram per kgm. and 66 per cent with 0.1 gram per kgm. of sodium salicylate. Sulfadiazine did not decrease the spreading effect of hyaluronidase.

In human subjects intradermal injection of the enzyme with the dye T1824 was said (17) to cause, in individuals with active or inactive rheumatic fever, unique reactions with enormous diffusion of the dye and local edema that sometimes involved the entire arm. Salicylate inhibited the spreading reaction in those cases. The type of reaction was also observed in one male with exanthematic typhus.

In this laboratory salicylate in vitro in equivalent or higher concentration has been found to be without effect on the depolymerization or hydrolysis of hyaluronate. However the marked depressing action on skin diffusion in rabbits has been confirmed (J. A. Coss, personal communication). The explanation of this effect as well as the beneficial action of salicylate on some rheumatic manifestations may be found in an inhibition of hyaluronate production of mesenchymal cells.

CONCLUSIONS

It is obvious from this review that the relationship between bacterial infection and the hyaluronidase system has continued to receive more of the attention

of investigators than has the metabolism of hyaluronic acid and its rôle in animal physiology and pathology. From the scanty data available, it seems obvious that the functions of the skin, of the ocular fluids, of synovial fluid and of the connective tissues in general must depend in part on the quantity and degree of aggregation of hyaluronic acid. Gels formed by the acid serve partly as the cement which holds cells together. In other structures as in the joint they protect internal surfaces, or they are part of the viscous barriers as in some connective tissues which regulate the exchange of metabolites and water. Thus the physiological aspects of hyaluronic acid as well as of other mesodermal cement substances seem to be of even greater importance than their rôle in infection.

REFERENCES

(1) Bauer, W., M. W. Ropes and H. Waine. Physiol. Rev. **20:** 272, 1940.
(2) Bensley, S. H. Anat. Record **60:** 93, 1934.
(3) Blix, G. Acta Physiol. Scand. **1:** 29, 1940.
(4) Blix, G. and O. Snellman. Ark. f. Kemi., Mineral. o. Geol. **19A:** no. 32, 1945.
(5) Blundell, G. P. Yale J. Biol. **14:** 373, 1942.
(6) Pøe, J. Acta Path. Microbiol. Scand. **21:** 587, 1944.
(7) Bungenberg de Jong, H. G. and W. A. L. Dekker. Kolloid Beihefte **43:** 213, 1936.
(8) Chain, E. and E. S. Duthie. Nature **144:** 977, 1939.
(9) Chain, E. and E. S. Duthie. Brit. J. Exper. Path. **21:** 324, 1940.
(9A) Clarke, R. W. Am. J. Physiol. **131:** 325, 1941.
(10) Cohen, S. J. Biol. Chem. **144:** 353, 1942.
(11) Crowley, N. J. Path. and Bact. **56:** 27, 1944.
(12) Duran-Reynals, F. Bact. Rev. **6:** 197, 1942.
(13) East, M. E., J. Madinaveitia and A. R. Todd. Biochem. J. **35:** 872, 1941.
(14) Favilli, G. Sperimentale Arch. biol. **89:** 724, 1935.
(15) Favilli, G. Nature **145:** 866, 1940.
(15A) Fekete, E. and F. Duran-Reynals. Proc. Soc. Exper. Biol. and Med. **52:** 119, 1943.
(15B) Glick, D. Ann. Rev. Biochem. **11:** 51, 1942.
(16) Guerra, F. J. Pharmacol. and Exper. Therap. **87:** 193, 1946. Arch. del Inst. de Cardiol. de Mex. **16:** 3, 1946.
(17) Guerra, F. Science **103:** 686, 1946.
(18) Haas, E. J. Biol. Chem. **163:** 63, 89, 101, 1946.
(19) Hahn, L. Ark. f. Kemi., Mineral. o. Geol. **19A:** no. 33, 1945.
(20) Hahn, L. Ark. f. Kemi., Mineral. o. Geol. **21A:** no. 1, 1945.
(21) Hahnel, E. and K. Meyer. Unpublished experiments.
(22) Hale, C. W. Biochem. J. **38:** 368, 1944.
(23) Hale, C. W. Nature **157:** 802, 1946.
(23A) Hechter, O. Science **104:** 409, 1946.
(24) Helferich, B. and A. Iloff. Ztschr. Physiol. Chem. **221:** 252, 1933.
(25) Hirst, G. K. J. Exper. Med. **73:** 493, 1941.
(26) Hobby, G. L., M. H. Dawson, K. Meyer and E. Chaffee. J. Exper. Med. **73:** 109, 1941.
(27) Hoffmann, D. C. and F. Duran-Reynals. J. Exper. Med. **53:** 387, 1931.
(28) Humphrey, J. H. Biochem. J. **37:** 177, 1943.
(29) Humphrey, J. H. Biochem. J. **37:** 460, 1943.
(30) Humphrey, J. H. J. Path. and Bact. **56:** 273, 1944.
(31) Humphrey, J. H. Biochem. J. **40:** 435, 1946.
(32) Humphrey, J. H. Biochem. J. **40:** 442, 1946.
(32A) Joel, C. A. and E. Eichenberger. Schweiz. Med. Wchnschr. **75:** 601, 1945.

(33) KABAT, E. A. J. Biol. Chem. **130:** 143, 1939.
(34) KASS, E. H. AND C. V. SEASTONE. J. Exper. Med. **79:** 319, 1944.
(35) KENDALL, F. E., M. HEIDELBERGER AND M. H. DAWSON. J. Biol. Chem. **118:** 61, 1937.
(35A) KURZROK, R., S. L. LEONARD AND H. CONRAD. Am. J. Med. **1:** 491, 1946.
(36) LEONARD, S. L. AND R. KURZROK. Endocrinology **37:** 171, 1945.
(37) LISON, L. Histochimie animale, Méthodes et problèmes. Paris, 1936.
(38) LURIE, M. B. AND P. ZAPPASODI. Arch. Path. **34:** 151, 1942.
(38A) MACCARDLE, R. C., J. P. BAUMBERGER AND W. C. HEROLD. Arch. Derm. and Syph. **47:** 517, 1943.
(39) MACLENNAN, J. D. Lancet p. 433, 1944.
(40) MACLENNAN, J. D. AND R. G. MACFARLANE. Lancet **2:** 301, 328, 1945.
(41) MADINAVEITIA, J. AND T. H. H. QUIBELL. Biochem. J. **34:** 625, 1940.
(42) MADINAVEITIA, J. AND T. H. H. QUIBELL. Biochem. J. **35:** 453, 1941.
(43) MADINAVEITIA, J. AND M. STACEY. Biochem. J. **38:** 413, 1944.
(44) MANN, I. C. The development of the human eye. London, 1928.
(44A) MANN, T. AND C. LUTWAK-MANN. Ann. Rev. Biochem. **13:** 25, 1944.
(45) MAXIMOW, A. Proc. Soc. Exper. Biol. and Med. **25:** 439, 1927.
(46) MCCLEAN, D. J. Path. and Bact. **34:** 459, 1931.
(47) MCCLEAN, D. J. Path. and Bact. **54:** 284, 1942.
(48) MCCLEAN, D. Biochem. J. **37:** 169, 1942.
(49) MCCLEAN, D. AND C. W. HALE. Biochem. J. **35:** 159, 1941.
(50) MCCLEAN, D. AND H. J. ROGERS. Lancet **1:** 707, 1943.
(51) MCCLEAN, D. AND H. J. ROGERS. Lancet **2:** 435, 1944.
(52) MCCLEAN, D., H. J. ROGERS AND B. W. WILLIAMS. Lancet **1:** 355, 1943.
(53) MCCLEAN, D. AND I. W. ROWLANDS. Nature **150:** 627, 1942.
(54) MEYER, K. AND E. CHAFFEE. Am. J. Ophth. **23:** 1320, 1940.
(55) MEYER, K. AND E. CHAFFEE. J. Biol. Chem. **133:** 83, 1940.
(56) MEYER, K. AND E. CHAFFEE. J. Biol. Chem. **138:** 491, 1941.
(57) MEYER, K., E. CHAFFEE, G. L. HOBBY AND M. H. DAWSON. J. Exper. Med. **73:** 309, 1941.
(58) MEYER, K., R. DUBOS AND E. M. SMYTH. Proc. Soc. Exper. Biol. and Med. **34:** 816 1936. J. Biol. Chem. **118:** 71, 1937.
(59) MEYER, K. AND E. HAHNEL. J. Biol. Chem. **163:** 723, 1946.
(60) MEYER, K. AND E. HAHNEL. Unpublished experiments.
(61) MEYER, K., E. HAHNEL AND R. R. FEINER. Proc. Soc. Exper. Biol. and Med. **58:** 36, 1945.
(62) MEYER, K., G. L. HOBBY, E. CHAFFEE AND M. H. DAWSON. J. Exper. Med. **71:** 137, 1940.
(63) MEYER, K. AND J. W. PALMER. J. Biol. Chem. **107:** 629, 1934.
(64) MEYER, K. AND J. W. PALMER. J. Biol. Chem. **114:** 689, 1936.
(65) MEYER, K. AND J. W. PALMER. Am. J. Ophth. **19:** 859, 1936.
(66) MEYER, K., E. M. SMYTH AND M. H. DAWSON. J. Biol. Chem. **128:** 319, 1939.
(67) MEYER, K., E. M. SMYTH AND E. GALLARDO. Am. J. Ophth. **21:** 1083, 1938.
(68) MORGAN, W. T. J. AND L. A. ELSON. Biochem. J. **28:** 988, 1934.
(69) NEUBERG, C. AND W. M. CAHILL. Enzymologia **1:** 22, 1936.
(70) PALMER, J. W., E. M. SMYTH AND K. MEYER. J. Biol. Chem. **119:** 491, 1937.
(71) PIRIE, A. Brit. J. Exper. Path. **23:** 277, 1942.
(72) RAGAN, C. Proc. Soc. Exper. Biol. and Med., in press.
(73) RAGAN, C. AND A. DE LA MATER. Proc. Soc. Exper. Biol. and Med. **50:** 349, 1942.
(74) RAGAN, C., E. HAHNEL AND K. MEYER. Unpublished experiments.
(74A) REID, M. E. Physiol. Rev. **23:** 76, 1943.
(75) ROBB-SMITH, A. H. T. Lancet **2:** 362, 1945.
(76) ROBERTSON, W. VAN B., M. W. ROPES AND W. BAUER. J. Biol. Chem. **133:** 261, 1940.

(77) ROGERS, H. J. J. Path. and Bact. **56:** 284, 1944.
(78) ROGERS, H. J. Biochem. J. **39:** 435, 1945.
(79) ROGERS, H. J. Biochem. J. **40:** 583, 1946.
(80) ROWLANDS, I. W. Nature **154:** 382, 1944.
(81) SCHWABACHER, H., A. C. CUNLIFFE, R. E. O. WILLIAMS AND G. J. HARPER. Brit. J. Exper. Path. **26:** 124, 1945.
(82) SEASTONE, C. V. J. Exper. Med. **70:** 347, 1939.
(83) SKANSE, B. AND L. SUNDBLAD. Acta. Physiol Scand. **6:** 37, 1943.
(84) SPRUNT, D. H. AND S. MCDEARMAN. Endocrinology **27:** 893, 1940.
(85) STEWART, W. A. AND K. MEYER. Unpublished experiments.
(86) VAUBEL, E. J. Exper. Med. **58:** 63, 85, 1933.
(86A) WERTHESSEN, N. T., S. BERMAN, B. E. GREENBERG AND S. L. GARGILL. Am. J. Urol. **54:** 565, 1945.
(87) WOLBACH, S. B. Am. J. Path. **9:** 689, 1933.

PATHOGENETIC FACTORS AND PATHOLOGICAL CONSEQUENCES OF DECOMPRESSION SICKNESS

H. R. CATCHPOLE[1] AND ISIDORE GERSH[1]

Naval Medical Research Institute, Bethesda, Md., and Department of Anatomy, The Johns Hopkins University, Baltimore, Md.

For almost fifty years no comprehensive review of the pathological consequences of decompression sickness has appeared. The reviews of Paul Bert (1878) and of Heller, Mager and von Schrötter (1900) defined the etiology of the disease and formulated the main aspects of its pathological results. The views of these authors have remained essentially unchallenged. But since that time a great clarification of the physical factors involved in aeroembolism has come about and a need has arisen to integrate more recent work in pathology with the older literature, with the objective of reinterpreting both in terms of these physical factors.

In dealing with this literature, key references have been preferred to exhaustive quotation. Deliberate limitations in subject matter have caused the exclusion from discussion of changes referable to the air-containing cavities, where air expansion *per se* gives rise to the pathological effect (pain, rupture, hemorrhage); thus removed from consideration are: aero-otitis media, sinus pain, pain and other effects arising from trapped gases in the intestinal tract, and, as possibly connected with the same phenomenon, pain associated with the teeth (86, 114, 129).

Some of these factors as pertaining to aviation have been dealt with by Armstrong (1). Also excluded are considerations of pathological physiological changes, e.g., in renal, cardiac and metabolic functions, in the gastro-intestinal tract, in the composition of the blood, in blood vessels and in the general phenomenon of shock. Effects due to anesthesia have been omitted, but a section on drugs and exercise is included. Effects directly referable to acute and chronic anoxia have also been omitted. In so doing, an effort has been made to separate the results of low oxygen on the body as a whole from those traceable to aeroembolism.

This review falls into two sections. In the first, physical and mathematical considerations governing the uptake and elimination of gases by the body and by individual tissues are discussed, and the conditions are defined for the relative susceptibility of a given site to bubble formation. In the second section, the gross and microscopical pathology of organs and tissues following decompression are related to these factors. While the effects of pressure and those of altitude are usually considered separately, the three categories of caisson disease, decompression sickness of divers, and aeroembolism of aviators, are regarded as basically similar entities, in which pathological differences are, or will prove to be, adequately explained by reference to the physical factors involved.

[1] Present address: Department of Pathology, University of Illinois, College of Medicine, Chicago, Ill.

PATHOGENETIC FACTORS IN DECOMPRESSION SICKNESS. *Physical Considerations. Mathematical principles.* The phenomena accompanying gas bubble evolution in the bodies of animals subjected to decompression have led recently to a revival of interest in the purely physical mechanisms involved in the separation of a gaseous phase from a saturated or supersaturated solution of a gas. This topic had already been explored in the eighteen seventies (for early literature, see 38, 72). The conditions for stability of a bubble of gas immersed in a liquid saturated with gas at 1 atmosphere have been defined (38, 72, 102) by the relation:

$$P = H + 2\sigma/r$$

where P = pressure in the bubble in excess of atmospheric, H = the hydrostatic pressure at the bubble level, σ = surface tension of the liquid, r = radius of the bubble.

As the radius r of the bubble diminishes, the second term of the above expression gets larger, and when r approaches the dimensions of a water molecule, the excess pressure due to the surface tension of the liquid is counted in thousands of atmospheres (38). There exists, in fact, a lower critical value for r (that is, a minimum bubble size) below which this excess pressure will literally squeeze the gas back into solution (72, 102, 128). Above the minimum size, however, the bubble will grow by diffusion as long as the tension of dissolved gas in its vicinity is greater than the gas tension in the bubble. For a free bubble to arise *de novo* it is necessary that this minimal size be achieved by sufficient molecules simultaneously attaining enough energy to overcome the forces of attraction between them. This was considered to be within the realms of statistical probability by Piccard (128). However, other theoretical treatments and experimental work reported by Dean (38) and Harvey (72) lead to the conclusion that bubbles do not tend to form in liquids spontaneously unless high negative pressures or considerable degrees of superheat are applied.

Gas masses existing in cracks or attached to irregular surfaces, on the other hand, have very different conditions for stability depending on the geometry of the surface, the shape of the gas-liquid-solid junction, contact angles, surface tension and ΔP (see below) which have been worked out for certain situations (72). Such gas masses may be stable at or below a critical size, but they grow indefinitely by diffusion above this critical size; an important property is that their gas content may be increased gradually by successive boostings. Gas nuclei are defined (72) as small invisible masses of gas usually, but not always, attached to a surface, which grow by inward diffusion of gas from the surrounding liquids. These enlarged masses may eventually become detached as free bubbles, or bubbles may become detached leaving behind a nucleus for the growth of other bubbles. The origin of these gas collections is obscure. The condition that they normally be attached to a surface, or contained in a crack, arises from the instability of small free bubbles noted above. Gas monolayers are believed not to promote bubble formation, but the possibility that multilayers of gas may act as nuclei has been suggested (38). Hydrophobic surfaces hold gases very tenaciously (72) but there is no evidence that tissue surfaces possess such properties.

Gas nuclei may be produced in surface cracks or acute angled cavities by statistical fluctuations of gas molecules (72, cf. also 128).

Harvey (71, 72) has introduced a useful expression to define the tendency, ΔP, of a gas to leave a liquid phase:

$$\Delta P = t - P$$

where t = gas tension in the liquid; P = hydrostatic pressure (this may be positive or negative in sign; the latter condition obtains when a pull is exerted on a liquid). Bubbles may form *de novo* when a very high negative pressure is produced in a liquid (when P is negative, ΔP increases). Acting in the vicinity of a gas nucleus, local negative pressures will favor the diffusion of gases into the nucleus. Such "cavitation" of a liquid may arise through pressure pulses, sound waves, Bernoulli effects (motion of liquids through constricted tubes), turbulent motion and through stretching. Increase of t will produce the same effect by providing a richer population of gas molecules for diffusion. Dean (38) attributed most cases of bubble formation in liquids in motion to vortices produced by turbulent flow. However, the streamlining of the vascular flow militates against this view as applied to bubble formation in animals (72).

Models of systems containing gas nuclei were studied by Pease et al. (126). Capric acid (M. P. 31°C) cooled to the point of crystallization provided foci for cavitation in aqueous solutions, and the process was reversed on warming; i.e., gas nuclei were apparently created and destroyed by these procedures. The same authors found that stearate monolayers on glass promoted cavitation; alcohols, amino acids and proteins abolished this effect. Removal of gas nuclei from liquids was early described by Tomlinson (cited by Dean, 38), and since that time bubble nuclei have been variously removed by boiling or partial evacuation of water, followed by standing (38); by centrifugalizing, filtering, boiling or subjecting to 1000 atmospheres pressure (72); and by pressure, evacuation or chemical agents (126). The remarkable properties of liquids freed from gas nuclei in resisting superheat and negative pressures have been frequently described (72, 97, 98). Harvey et al. (73) distinguished between macronuclei, removable from water by centrifugalization, and micronuclei, removable only by high pressures (1090 atmospheres). Freshly drawn blood is completely free of macro- and micronuclei; such nuclei are neither present in the blood plasma, nor attached to formed elements or to any other constituent of the blood. It must therefore be assumed that they are attached to the linings of blood vessels, and that bubbles formed at the sites of the nuclei are released into the circulation (72).

Factors in the growth of gas nuclei in the body. If the concept of $\Delta P = t - P$ be adopted as a measure of the bubble forming tendency, and the presence of gas nuclei in the body be accepted, a number of conditions favoring, or tending to prevent, bubble formation become intelligible. Local production of CO_2 increases t and therefore increases ΔP, and so conduces to bubble appearance (69, 110); muscular activity, besides increasing the CO_2 tension locally gives rise to mechanical tensions and to consequent decrease in P; for both reasons it favors

bubble formation (28, 68, 110, 177). Tissue manipulation of almost any kind (stretching, cutting, crushing) leads to the same result (73). Bone fractures are a potent site of bubble formation (17, 70), since they lead to sharp momentary falls in the value of P. Bubbles form more readily in veins (low P) than in arteries (high P) (72, 177). Among factors delaying or preventing bubble formation in cats stimulated to muscular activity at altitude were: pre-stimulation (109, 177), anoxia (109, 69), traumatization of legs by skinning (109); these treatments promote local hyperemia or hyperventilation, and result generally in an increased value for P combined with faster elimination of nitrogen. Not susceptible of such simple analysis are the effects of vasoconstriction, which are equivocal, and of vasodilatation (aminophyllin), which are negative (137). It is evident that for the body as a whole, ΔP represents an aggregate of pressure differences of constantly varying magnitude in different sites. The situation is summarized by Harvey (72): "at ground level ΔP is nearly zero in tissues to negative in arteries. On rapid ascent to high altitude it is at first positive everywhere, but quickly becomes negative in arteries (due to blood pressure and rapid equilibration with alveolar air), remaining locally high in small vessels (due to CO_2 and N_2 of tissues) for a time, finally becoming zero to negative except for regions of fat deposits or very poor circulation."

Establishment of critical pressure differences. Decompression establishes ΔP values necessary for bubbles to grow. Boycott, Damant and Haldane (21) found experimentally in goats and man that decompression from 2.3 atmospheres to one atmosphere never produced symptoms. On the basis of equivalent gas volumes they argued that a drop of approximately one-half of the original gas pressure would be safe whatever its value (e.g., from 4 to 2 atmospheres, from 6 to 3 atmospheres, etc.). But as will be seen in a succeeding section, this assumption is untenable, and Haldane himself (65) has stated that above six atmospheres it is no longer quite safe to halve the initial pressure. Behnke (5) proceeded on the basis that the difference between tissue gas pressure and the external pressure should at no time exceed 1.3 atmospheres (2.3 minus 1.0 atmosphere). He then calculated decompression rates that would hold the external pressure at not more than 15 lb/sq. in. below the greatest tissue pressure. The views of both Haldane and Behnke have been critically examined by de Burgh Daly and his associates (37, 43). Smaller animals appear to be able to tolerate greater pressure differentials. Guinea pigs survive decompression from 60 lb/sq. in. (gauge) to atmospheric pressure (15 lb/sq.in.), and bubbles are produced with difficulty in rats and mice.

For the occurrence of aeroembolism in man there appears to be a critical altitude of 20,000 to 25,000 feet (0.45 to 0.37 atmosphere) as given by the British authorities (133), or perhaps somewhat higher. Under extreme conditions of stimulation, bubbles appear in cats decompressed to 35,000 feet (0.23 atmosphere). In rabbits, bubbles can not usually be produced at 40,000 feet (0.18 atmosphere); at 45,000 feet they are formed under specified conditions (27). Bubbles scarcely appear in quiet frogs below 60,000 feet, although violent mus-

cular activity reduces the ceiling considerably (177). The smaller rodents again are relatively refractory to bubble formation and rats at altitude, in a state of normal activity, do not yield bubbles at 50,000 feet (177).

Effect of rate of decompression. Since the time of Paul Bert, it has been repeatedly demonstrated that the severity of decompression sickness is directly related to the rate of decompression. Altitude studies by Griffin et al. (62) compared the effect of different rates of ascent (1000 and 5000 ft. per minute) and showed a markedly greater susceptibility to bends after the faster ascents.

Systematic studies on rabbits decompressed to altitude showed a relation of rate of decompression to the incidence and severity of bubble formation (table 1) (27). When decompressed to 45,000 feet in eight minutes or less, mortality was high, and survival time became progressively shorter as the time to altitude decreased. At decompression times exceeding ten minutes to reach 45,000 feet, mortality was low and bubble incidence minimal or zero.

TABLE 1

Effect of rate of decompression on the 50 per cent survival time, the percentage of deaths, and the symptoms of rabbits decompressed to 45,000 ft.

RATE OF DECOMPRESSION	50 PER CENT SURVIVAL TIME	PERCENTAGE OF DEATHS	AVERAGE BUBBLE INCIDENCE SCALE: 0 (NO BUBBLES) TO 5 (MOST NUMEROUS) (27)
	min.		
3–10 sec.	12	81	5 to 2
30 sec. to 1 min.	7	92	3 to 1
2 min. to 5 min.	13	66	2 to 0
6 min. to 8 min.	17	80	1 to 0
10 min.	30	15	0

Similar considerations appeared to control the death of animals receiving injections of air at varying rates into peripheral vessels (140).

Decompression from high pressures and to altitude. The principle that decompression from five atmospheres to one is far more hazardous than from one atmosphere to one-fifth of an atmosphere was demonstrated *in vitro* by Piccard (128). Water saturated with air gave brisk effervescence in the former case, and slow, delayed evolution in the latter. The total *volume* of gas available for release was the same in both instances. The explanation (68, 128) lies in the greater *weight* of gas dissolved at the high pressure, and consequently the greater number of gas molecules available for diffusion into gas nuclei (or for the formation of aggregates of gas by collision). This determines the rapidity of bubble formation, when the pressure is suddenly released. An *in vivo* counterpart of this demonstration is shown by a comparison of bubble frequency and distribution in guinea pigs decompressed from 105 lb/sq. in. gauge pressure to atmospheric pressure, and in rabbits decompressed from ground level to 45,000 feet (51). Bubble formation was far more severe in the former case (table 2) although the relative pressure reduction was seven to one in both. These results are entirely

similar to those reported by de Burgh Daly et al. (37, 43) who compared the results of decompressing rabbits, guinea pigs and rats from 6.3 to 1.0 atmosphere, and from 1.0 to 0.16 atmosphere. It is clear that the reduction in pressure by one half employed by Boycott, Damant and Haldane (21) would actually become more hazardous as the initial pressure increased.

Composition of gas bubbles. It was conclusively shown by Paul Bert (18) that nitrogen formed the major constituent of gas bubbles recovered from animals after decompression from compressed air atmospheres. The rôle played by CO_2 in bubble formation has given rise to some speculation as to the composition of gas bubbles at the site of formation. Gas diffusion constants for the common respiratory gases are approximately equal, and the composition of a bubble will

TABLE 2

Comparison of the distribution of gas bubbles in rabbits decompressed to a simulated altitude of 45,000 feet with guinea pigs decompressed from compressed air at 105 lb. per sq. in. (gauge)

	HIGH PRESSURE	LOW PRESSURE
Extravascular gas bubbles		
Adrenal	Numerous	None
Nerves	Numerous	None
Fat (intracellular)	Numerous	None
Blood vessels		
Arteries and veins	Present	Present
Capillaries	Present	Fat only
Spleen		
Sinusoids	Present	Present
Arteries and veins	Present	Present
Branches of pulmonary arteries	Present in many	Present in few
Liver		
Sinusoids	None	None
Central vein	Few	Few
Liver cells	Watery vacuoles present	No watery vacuoles present
Intestine	Numerous	Few
Muscle	Numerous	Few

therefore be largely controlled by the amount of gases close to it (38). Carbon dioxide is some 50 times more soluble in water than nitrogen. In CO_2 rich regions, such as a contracting muscle, this gas may condition the formation of the primary bubble and represent its principal constituent, at least for a while. Recent experiments (69, 110) support the rôle of CO_2 as a facilitator in bubble formation. When the bubble rich in CO_2 is moved to a body region rich in nitrogen and poor in CO_2, the latter gas will diffuse out and nitrogen in, to give bubbles essentially composed of nitrogen.

Gas Uptake and Elimination. Mathematical principles. Since the first demonstration of the importance of nitrogen in events leading to decompression sickness, attempts have been made to analyse experimentally and describe theoretically the course of gas uptake or elimination when the ambient pressure is raised or

lowered. That gas uptake would present the characteristics of a logarithmic relationship was recognized by Zuntz (185) and by Heller, Mager and von Schrötter (80). Employing the findings of Vernon (168) on the high solubility of nitrogen in fat, Boycott et al. (21) also presented a logarithmic relationship, but indicated a slower rate of saturation per round of circulation than the above authors. They stated further that their computations gave a rough approximation only to the actual rate of saturation for the body as a whole, due to variations in blood flow to the several tissues and to differences in body composition. Experimentally they found in goats a 94 per cent saturation in 3 hours. From this they deduced a similar degree of saturation for man in 5 hours. In computing decompression tables for divers, they arbitrarily assumed the existence of tissues having half saturation times of 5, 10, 20, 40 and 75 minutes, while admitting the possibility of tissues saturating at yet slower rates. Campbell and Hill (23) showed that about one third of the gaseous nitrogen of the human body was removed in the first few minutes while breathing 100 per cent oxygen, but some tissues were found to remain unsaturated after several hours' excess pressure (24). Hawkins, Shilling and Hansen (76) from a study of a large series of experimental dives modified the British decompression tables. They also adhered to the assumption of tissues having half saturation times of 5 to 75 minutes. Decompression times were radically reduced, especially for dives of short duration, in which the "slow" tissues would have become only partially saturated.

That saturation and desaturation curves should be reciprocal was indicated by Boycott et al. (21) and shown experimentally by Shaw et al. (146). The latter further showed that nitrogen absorption obeys Henry's law. A somewhat different concept was introduced by Behnke and his co-workers (5, 6, 7, 8, 12). Total nitrogen of the body was considered to be partitioned between aqueous and fatty phases, and the curve of nitrogen elimination was represented as the sum of two exponential expressions governing respectively the "water" nitrogen and the "fat" nitrogen. There was satisfactory agreement between nitrogen elimination found for man, and that calculated from the equation:

$$Y = \underset{\text{(water)}}{382\,(1 - e^{-0.0085\,t})} + \underset{\text{(fat)}}{458\,(1 - e^{-0.098\,t})}$$

where Y = total elimination of N_2, t = time in minutes, e = base of natural logarithms (12).

Some question was expressed subsequently of the adequacy of the k values cited above. Underwoood and Diaz (164) injected radon into the saphenous vein of the dog and studied its elimination through the lung at one minute intervals for four minutes. Part of the gas in the body was shunted to regions of poor circulation and was not measured in the short time interval allowed. They found k values of 0.66 as compared with the much smaller values obtained by Behnke (12), and believed that their figure applied to gas leaving the blood whereas the Behnke values were for gas entering the blood from tissues. This finding was held to resolve the difficulty encountered by Shaw et al. (146) which led to their postulation of a peculiar state of nitrogen supersaturation *in vivo*.

Smith and Morales (150, 151), Morales and Smith (117, 118, 119), Jones et al. (90), and Ferris et al. (48) have contributed further to the analysis of blood-tissue gas exchanges and it becomes necessary to establish the general trend of such attempts to date. It appears to be implicit in the work of Boycott et al. (21) that the total body nitrogen reservoir may be represented as the sum of 5 (or more) arbitrary "tissues" with half saturation times of 5 to 75 minutes. Shaw et al. (146) and Behnke et al. (12) also considered that nitrogen elimination could be represented empirically by one or more exponential equation of the form

$$Y = A\ (1 - e^{-kt})$$

where Y = amount of N_2 eliminated in time t (minutes), A = amount of N_2 originally present, k = constant of elimination, e = base of natural logarithms.

In actual practice two such functions were used and referred to water and fat phases respectively. Underwood and Diaz (164) generalized from the work of these latter authors to the form

$$Q = \Sigma_i Q_0\ (1 - e^{-k_i t})$$

where Q = total amount of gas eliminated, t = time in minutes, ${}_iQ_0$ = initial amount of gas present in the i state (i.e., in water, fat, etc.), k_i = elimination constant for the i state, e = base of natural logarithms.

Smith and Morales (150) in developing an equation for the uptake of inert gas by tissue regions, e.g., the tissues of a limb, considered the following physical and physiological factors to be operative in gas exchange: blood volume of the region, delivery rate of blood flow, delivery concentration of gas in the blood, tissue volume, gas solubility in each tissue, area of the capillary bed, and tissue permeability to the gas. They derived an expression for tissue regions identical in form with that of Underwood and Diaz (164) but in which the various Q and k values were invested with physiological meaning. These quantities are not to be considered as characteristics of a specific tissue component (water or fat, etc.), but are functions of all tissues of the region, and of the circulation. They concluded (117) that the early, more rapid absorption stage is governed by the blood and by aqueous tissues in close relation to it, and the slower, later stages predominantly by fatty tissues; that these processes are nevertheless simultaneous and conditioned by physical and physiological factors whose importance varies from tissue to tissue and from region to region. These factors may be varied, e.g., by the substitution of one inert gas by another, by increasing blood flow to a region and by altering the degree of fatness. By the method of deriving the general equation employed by these authors, the effects of altering these variables are claimed to become predictable. Further, these quantities may be measured independently of gas uptake, and substitution into the equations developed gave agreements held to be close enough to justify the method of approach (117). Jones et al. (90, cited 117) found curves for nitrogen desaturation of the whole body to conform to the general equation. They emphasize in particular the rôle of the circulation, and believe that all their results are explainable on the basis of gas solubility and blood-tissue perfusion rates.

Gas solubilities. The greater susceptibility of fat individuals to decompression sickness was noted by Boycott and Damant (20) who deplored the fact that increase in experience and technical skill should so often be associated with the increasing waist that accompanies the onset of middle life. This susceptibility was attributed to the greater solubility of nitrogen in fat which becomes a gas reservoir maintaining nitrogen pressure long enough for bubbles to form during the pressure drop of decompression. From this time, considerations of gas solubility in body constituents have occupied an important rôle in the theory and practice of decompression sickness. It is generally held that tissues, with the exception of fat, take up gases in proportion to their water content (23). Data on the solubility of gases in plasma and body fat are incomplete, but their solubility in water and certain oils is better known. Since the effects of salts and proteins (74, 75, 144, 167) and the differences in the nature of the lipids (168)

TABLE 3

Solubilities, relative solubilities and ratio of solubility in oil to solubility in water of respiratory and inert gases presented in order of ascending molecular weight and density. Cited in part from ref. 161

GAS	MOL. WT.	DENSITY AIR = 1.0	SOLUBILITY IN WATER AT 37°C.	RELATIVE SOLUBILITY IN WATER He = 1.0	SOLUBILITY IN OIL AT 37°C.	RELATIVE SOLUBILITY IN OIL He = 1.0	RATIO OF SOLUBILITY IN OIL TO SOLUBILITY IN WATER
			cc/100 cc		*cc/100 cc*		
H_2	2.0	0.0695	1.6	1.9	4.5	3.0	2.8
He	4.0	0.138	0.85	1.0	1.5	1.0	1.7
N_2	28.0	0.963	1.3	1.5	6.7	4.5	5.2
O_2	32.0	1.105	2.4	2.8	12	8.0	5.0
A	39.9	1.38	2.6	3.1	14	9.5	5.4
CO_2	44.0	1.529	56.0	66.0	87.6	58.0	1.6
Kr	83.7	2.868	4.5	5.3	43	29	9.0
Xe	131.3	4.525	8.5	10.0	170	110	20.0
Rn	222	7.526	15	17.5	1900	1300	126.0

on gas solubility are relatively small, the values for water and oil may be used as first approximations for plasma and fat (12, 23, 168). Oil and water solubilities of H_2, O_2, CO_2, N_2 and of the inert gases He, Ne, A, Kr, Xe, Rn have been recently critically examined and tabulated (161). Values for some members of the series were newly derived using a radioactive isotope technique, while other values were derived from the older literature. Some of these results are included in table 3.

Nitrogen. If the fat content of the body is 15 to 20 per cent, the fivefold greater solubility of nitrogen in fat compared with water implies that one-half of the total nitrogen of the body is dissolved in fatty tissue (5, 12, 21, 23). During short exposures to excess pressure, fat may act as a reservoir to protect the body against sudden flooding with bubbles (5). Times for essentially complete saturation of the body by nitrogen are given as: man, 5–6 hours (20, 23); dog, 3–4 hours (146); goat, 3 hours (21). Small animals saturate and desaturate much

more rapidly (21) and require higher pressure differentials to elicit bubble formation.

Oxygen. In atmospheres of compressed air, the amount of dissolved oxygen in tissues represents an inconsiderable part of the dissolved gases. However, the oil-water ratio for oxygen approximates that for nitrogen, and exposure to 100 per cent oxygen under pressure followed by decompression leads to the appearance of gas bubbles (53, 81, 83). For reasons believed to involve tissue damage by oxygen under pressure, bubble formation was of less intensity on decompression from oxygen atmospheres (53). In decompressions to altitude from oxygen at atmospheric pressure the formation of bubbles is greatly reduced, and pretreatment with oxygen is commonly employed for protection under these circumstances (28, 49, 177, 179).

Helium. A low oil-water ratio and the low solubility in both plasma and fat has rendered this gas relatively ideal as a diluent for oxygen in diving (6, 13, 15). The total body content of He at saturation is only 40 per cent that of N_2 and relatively less is present in the "slow" tissues. Time for helium elimination is approximately 50 per cent that required by nitrogen. There is a cutaneous diffusion of helium of 50 cc. per hour which increases rapidly above 28°C with the concurrent sharp rise in peripheral blood flow (14).

Hydrogen. The solubility characteristics of hydrogen are less favorable than those of helium and no special advantage is gained by its use. It has been employed in experimental diving work (183).

Argon, Xenon, Krypton, Radion. The oil/water solubilities of these gases increase roughly in proportion to their atomic weights (91). The last three have found application chiefly as radioactive tracers. Use of argon as a diluent for oxygen in compression studies has served to confirm the view that the production of a highly saturated reservoir of inert gas favors bubble formation. Symptoms of increasing severity were produced by gas mixtures in the following order: oxygen, helium-oxygen, air, argon-oxygen (53), conforming to the oil/water solubilities of helium, nitrogen and argon respectively.

Chemical composition of the body. Arbitrary resolution of the body into lean body mass and fat in studying abnormal pressure effects was suggested by Behnke (6, 9) and a theoretical formulation was developed by Morales et al. (115). The lean body mass, consisting of bone and other tissues, together with essential lipids, maintains in the adult a relatively constant composition. Body density was used as an index of obesity by Behnke et al. (10). By comparing body specific gravity with direct estimations of total fat in the guinea pig, Rathbun and Pace (134) formulated a quantitative relationship between them. A similar relation between specific gravity and fat content was suggested by these authors for man. Computations of body composition from inert gas uptake have been made (6) and methods involving the differential uptake of two inert gases have been suggested as a means of measuring watery and fatty tissues (91, 116, 123) but apparently have not been elaborated experimentally.

Analysis of the effects of decompression on individual tissues requires a breakdown of their composition in terms of fatty and non-fatty components. Values have been derived from the literature for the composition of some tissues of

particular interest in decompression (table 4). The fat content of bone marrow is high (56, 85, 173). Since bone marrow may represent from 2.2 to 4.4 per cent of the body weight, bone marrow fat may comprise up to one-fifth of the total body fat. The adrenal gland is relatively rich in fat (7.5–14 per cent) and in decompression from high pressures a fall in specific gravity of this tissue has been correlated with the presence of extravascular bubbles, paralleling the situation existing in fat tissue proper (52). Brain (156), nerve, liver and muscle are relatively poor in fat; the skin, as a rule, is somewhat richer than these tissues (66).

Tissue blood flow. Inert gas uptake depends among other things on the rate of blood flow, and heavily perfused organs saturate and desaturate rapidly (150). It may be assumed that urine originating in Bowman's capsule would reflect the gas tension of the glomerular blood. Hill and Greenwood (81) early showed experimentally in man that the pressure of nitrogen in the urine became equal to

TABLE 4

Fat content of body tissues and organs

TISSUE OR ORGAN	ANIMAL	FAT CONTENT		REFERENCE
		Per cent wet weight	Per cent dry weight	
Brain	Man	5–8		156
Yellow bone marrow	Pig	87–90		24
	Ox, sheep, horse	90–96		24
	Goat	90–95		24
Red bone marrow	Rabbit	70–90		173
Adrenal gland	Guinea pig	7.5–14 (calculated)	30–54	52
Nerve-sciatic	Guinea pig	5.1 (calc.)	10–24	52
Liver	Guinea pig	1.4 (calc.)	4.9 (range: 0.5–11.8)	52
Muscle	Guinea pig	1.7 (calc.)	6.8 (range: 1.0–16.5)	52
Skin	Rat	6.6–12		66

that in the alveoli within 10 minutes after exposure to increased pressure and that the excess nitrogen rapidly disappeared when the pressure was lowered. Behnke and Yarbrough (15) found that the saturation time for urine in men breathing a He-O_2 mixture was between 30 and 60 minutes, and desaturation time occupied the same interval in the absence of bubble formation. In the latter case, desaturation was delayed. It would be expected that organs with perfusion rates of a comparable order, such as the thyroid and the liver would saturate and desaturate with equal rapidity.

Values for blood flow through tissues and organs have been derived from the literature (table 5). Classical methods of measuring blood flow were used in most instances. However, Jones et al. (90) have calculated blood-thyroid and blood-liver perfusion rates from the exchange of radio-iodine and radio-phosphorus, respectively. Tissues with poor blood supply include fat, bone marrow and resting muscle; active skeletal muscle, heart muscle and brain have a moderate

blood supply and the kidney and thyroid have the richest blood flow. Lying outside this general classification are organs like the spleen with an intermittent circulation and the liver with its dual circulation.

Local peculiarities of the circulation may modify figures based on the total perfusion rate. Gersh (56) contrasted the intermittency and probably reduced rate of flow in splenic sinusoids with the relatively continuous flow in those of the liver. Hepatic blood flow has been estimated to be from one-third to one-seventh of the portal blood flow of the liver (63, 152). In decompression, the slower and presumably more saturated portal blood becomes diluted with less saturated blood from the hepatic artery, thereby lowering the tension of gas available for bubble formation. Organs having blood trapped in sinusoids may on the other hand be exposed to sharp pressure differences between the entering arterial blood

TABLE 5

Blood flow in tissues and organs

TISSUE OR ORGAN	ANIMAL	BLOOD FLOW	REFERENCE
		ml/1000g/min.	
Thyroid	Man	4000	90
	Mouse	3240	90
Kidney	Man	3000	90
	Man, dog, rabbit	3400, 3300, 3200	148
Brain	Monkey	850 (range: 600–1000)	40
	Man	650 (range: 460–1040)	142
Heart	Dog	500–750	47
Muscle (dilated)	Dog	57–570	124
(dilated)	Dog	110–580	178
Muscle	Man	24 (range 5-rest to 50-exercised)	90
	Dog	69 (range 30–130)	61
Bone marrow	Goat	26	24
Fat	Man	12–15	90

and the trapped sinusoidal blood, sufficient to produce extravascular or intravascular bubbles. The rapid saturation of all structures of the kidney related to the glomerular apparatus may not extend to portions of the renal tissue having a different vascular pattern.

Capillary density and surface. While descriptive accounts of the capillary distribution in tissues abound, quantitative studies relating capillary surface (S) to unit tissue volume (V) are largely lacking. Values for total and open capillaries of fat tissue were derived (54) from histological studies on injected and frozen-dried preparations respectively. The ratio S/V (cm^{-1}) was computed for fat-rich and fat-poor adipose tissue and compared with estimates for muscular tissue based on data provided by Krogh (101) (table 6). Open capillaries represented from one-half (fat-rich) to one-quarter (fat-poor) of the total capillary area of fat; a similar general relationship exists in muscle (147). For other tissues,

estimates of S/V by indirect means are probably subject to considerable error. Values have been given (147) for the number of red blood corpuscles per cubic millimeter of tissue, which may be regarded as a rough measure of the number of open capillaries. Accepting the fairly reasonable value of 1000 cm^{-1} as a measure of S/V of the capillaries of mouse heart muscle, values for some other tissues have been computed (table 7). Fat-rich adipose tissues have available for gaseous exchange one quarter or less surface than the most poorly supplied muscle, and fat-poor tissues barely equal the poorest muscle. The relative in-

TABLE 6

Capillary surface of tissues in relation to total tissue volume (S/V)

TISSUE	ANIMAL	s/v (cm^{-1})		REFERENCE
		Total (open plus closed capillaries)	Open caps.	
Fat Fat-rich	Rat	51.9	23.5	54
Fat-poor	Rat	222.2	64.1	54
Muscle (a)	Rabbit	190–513		42
(b)	Rabbit	150–295		153
(c)	Dog	494		101
(d)	Guinea pig		186–507	174
(e)	Mouse		486–923	174
Cardiac muscle	Man	1184		175

TABLE 7

Relative values of S/V calculated from the number of red blood corpuscles per c.mm. of tissue as estimated by Sjöstrand (147)

TISSUE	ANIMAL	NO. CORPS./c. mm.	s/v (cm^{-1})
Heart muscle	Mouse	350	1000
Brain-cortex	Mouse	85	240
Cerebellum	Mouse	115	330
Liver	Mouse	280	800
Kidney-cortex	Mouse	300	850
-medulla	Mouse	500	1400
Duodenum	Mouse	160	460
Pancreas	Guinea pig	125	360

adequacy of the capillary bed of fatty tissue is therefore demonstrated quantitatively (54).

Cerebral cortex and cerebellum compare unfavorably with muscle in respect of the capillary bed available for gas transfer. Cobb and Talbot (34) and Cobb (33) concluded that even the most vascular parts of the brain were only one-fifth as vascular as skeletal muscle. Within the central nervous system itself Craigie (35), from measurements of the total length of capillaries in sections of the central nervous system of the cat found the poorest part of the grey matter to be one and one-half times as richly vascularized as the richest part of the white matter. The same was true in the monkey. In the cat Wolff (181) found the

following relative vascularities: parietal cortex, 100; lateral geniculate ganglion, 91; globus pallidus, 58; nerve, 47; and white matter, 43. Thus the white matter is something under one-half as richly vascularized as the grey. In addition, capillaries in the white matter are said to be larger than those of the grey (33) which would make S/V values for the white matter even more unfavorable.

Occupying a somewhat intermediate position in the S/V scale is skeletal muscle which possesses nervous and humoral mechanisms capable of rendering effective large portions of the total capillary surface when active, i.e., at those times when the conditions for gas bubble formation are apt to approach the critical. Highest in the scale are cardiac muscle, kidney and liver which appear to possess a large effective capillary surface for gaseous exchanges that is available at all times.

PATHOLOGIC CONSEQUENCES OF DECOMPRESSION SICKNESS. *Central Nervous System. Pressure.* Little can be added to the classical description by Heller, Mager, and von Schrötter (80) of the neurological aspects of the syndrome of decompression sickness. The substance of their detailed description follows: After a latent period, symptoms of varying degrees of severity may appear. These may be general in scope (syncope, dizziness, aphasia), may include large body masses (paraplegia and paralysis of both lower legs, of both arms, of one arm and one leg, or both legs and one arm with a persistent spastic paralysis, loss of urinary and intestinal sphincter control), may include sensory paresthesias and hypo- or analgesia separately, or associated with motor disturbances, or may be confined to small motor or sensory units (paralysis of the left lateral rectus muscle, a single orbicular muscle, or the hypoglossal muscles on one side, labyrinthine deafness, or atrophy of the left optic papilla). Neural damage due to aeroembolism thus varies greatly, is non-specific, and unpredictable: 1, it may be extensive, covering large regions, or confined to minute structures; 2, it may be largely motor with a sensory component, or the latter may be dissociated; 3, it may be confined to areas supplied by cranial nerves or somatic nerves.

The pathologic changes in the central nervous system following damage by aeroembolism have been studied extensively in man and other animals ever since 1870. Because of numerous precautions taken to reduce the incidence of decompression sickness and because of the success of treatment when such accidents occur, the most satisfactory pathologic studies date back about half a century. The most prominent feature is the occurrence of areas of softening in the spinal cord. Although they may extend over the greater part of the spinal cord (18), they are most commonly limited to the thoracic cord (19, 29). They are less frequent in the upper lumbar segments (80, 184) and are rather infrequent in the cervical region (21, 80, 166, 184). The areas of softening are usually small and are more circumscribed in the white than in the gray matter. The small necrotic areas may fuse, and result in an apparent general softening in large regions (80). Necrosis is more common in the white than in the gray columns (80), and in the former, is most frequent in the anterior columns (21). The ascending degeneration of nerve tracts anterior to the site of multiple lesions, and the descending degeneration posterior to the region have been described by numerous pathologists (19, 80, 143, 145).

Heller, Mager and von Schrötter have described the individual lesions most

completely. In the necrotic regions of the white matter, the nerve fibers swell, degenerate and are completely resorbed. The glial meshes are spread during the edema which takes place. Sclerotic areas appear, radiating into the surrounding, less damaged nerve fibers. The cavity enclosed contains cellular detritus and myelin droplets, and some phagocytic cells. The cavity then becomes more regularly outlined and the walls of the surrounding blood vessels become thicker. In the gray matter, the same processes take place, but in the surrounding regions, nerve cells in all stages of chromatolysis may occur. The necrotic cavity is not as sharply outlined in the gray as in the white matter.

While hemorrhage may occur, it is infrequent and is definitely less prominent than the regions of necrosis described above (18, 19, 80, 171, 184). It may, however, be of greater significance when it occurs in the brain (18, 29) or in the inner ear (80).

Gas bubbles have been described in pial vessels of the spinal cord, medulla and cerebrum in autopsied men, and in other animals (18, 21, 29, 80, 122, 184). As it is difficult to ascertain whether these bubbles were present where described at the time of death, the observations of Pudenz (132) and of Wagner (172) are of greater significance. The former observed in a monkey the appearance of gas bubbles first in pial arteries and then in veins in cinematographs of the cortex exposed to view by the use of a lucite calvarium. The latter made essentially the same observations in a series of cats whose pial vessels were viewed continuously before, during, and after decompression through a Forbes window. A gas bubble was observed also in the ophthalmic artery of a dog (29). Also more reliable than the earlier accounts are the preliminary observations of Gersh, who preserved the sites of gas bubbles by the freezing-drying method. Gas bubbles were found in the spinal cord of guinea pigs directly after massive aeroembolism *only* in the blood vessels; they were present also in intraneural blood vessels of the sciatic nerve. There was no evidence to support the assertion of Boycott et al. (21) that gas bubbles occur extravascularly in the central nervous system. Finally, minute gas bubbles confined entirely to the myelin sheath were present in peripheral nerves of guinea pigs decompressed from high pressure atmospheres of argon-oxygen, air, helium-oxygen, and oxygen (53).

The general pattern of neurologic symptoms, pathologic changes and the distribution of gas bubbles in the central nervous system following decompression was outlined by Bert (18). It was confirmed and extended by the magnificent work of Heller, Mager and v. Schrötter (80) which the authors have leaned on heavily, and by later workers. Gas bubbles form in the circulating blood after a short latent period following decompression. Most bubbles are filtered out by the lungs; some bubbles, however, pass through the lungs, either by means of small arterio-venous anastomoses which may be assumed to exist, or through the capillary "lakes" of Sjöstrand (147). These bubbles are small, about 25 μ in diameter. When they pass through the heart and reach the central nervous system, they occlude small arterioles of the same order of magnitude (21, 52, 132, 172). Since the circulation in the central nervous system is largely terminal in nature, the venous blood flow and blood pressure in the region is reduced, and

gas bubbles appear secondarily in the veins. Another consequence of the terminal nature of the circulation is that nerve cells and fibers in the affected region are suddenly deprived of their major source of nutrients and oxygen. They rapidly degenerate in small, focal regions corresponding to the arterioles occluded by gas bubbles. The necrotic regions may be separate, or may fuse secondarily with adjacent foci to form larger areas of softening. The extreme rapidity with which nerve cells are irreversibly injured following occlusion of the circulation has been conclusively demonstrated by numerous workers (58, 92, 93, 94, 176, 180). The unpredictability of the site and extent of the lesion, as well as the magnitude of the symptomatology, are thus related to the site of lodgement of the gas emboli, which may be isolated or adjacent to others, and to the numbers of blood vessels occluded. It cannot be stressed too strongly that there is a large element of chance as to whether a given number of gas bubbles causing neural damage will result in minor or severe symptoms, depending on the nerve cells and fibers affected. The circulation in the gray matter of the spinal cord is somewhat anastomotic, and this may account for the fact that necrotic foci occur less frequently in this portion of the spinal cord than in the white matter. Another circumstance which protects the gray matter is the fact that the capillary density, and presumably the blood flow, is greater than that in the white matter (35).

Hemorrhage is a relatively unimportant factor in neural damage of decompression sickness. Although v. Leyden believed that expanding extravascular gas bubbles induced hemorrhage by laceration of blood vessels (171), the same result may take place due to the occlusion and weakening of small blood vessels. It is also possible that vascular congestion somewhat remote from the gas bubble and the cessation of the blood flow may result in hemorrhage. This was actually observed directly in mesenteric vessels of animals injected with small gas emboli (30, 162).

The appearance of minute gas bubbles in the myelin sheath of peripheral nerves after decompression is attributable to the large volume of gas in solution in the lipid phase of the myelin sheaths. While the lipid content of the brain is only 5–8 per cent (156), certain parts may be expected to be richer in lipid. It is on this account that the earlier English investigators attributed the alleged occurrence of extravascular gas bubbles to the release of the excess lipid-soluble gas (21). Apart from the necessity for confirmation, the conclusions may be modified as a result of the later work by Campbell and Hill (23), who showed that the brain of pressurized animals contains far less gas than expected. This discrepancy in gas uptake may possibly be explained by further knowledge of blood flow and capillary density in the brain. Similar investigations may aid in interpreting several other problems without a satisfactory solution: the predominance of lesions of the spinal cord as compared with the brain, the marked predilection for injury of the thoracic cord, and the relative severity of damage to the motor systems as compared with the sensory.

Altitude. Symptoms of damage to the central nervous system following decompression to altitude are rather uncommon, and pathologic changes are even

rarer. There is no evidence that lasting changes occur in the cerebral cortex (45), in spite of the appearance of irregular EEG waves with a low frequency and high voltage during or after decompression to extreme heights (182). No residual EEG changes were detected in individuals decompressed to 35,000 feet even while they were suffering from the chokes or the bends. In subjects free from the effects of *generalized* shock, some temporary damage to the higher centers may occur, for there may be temporary syncope, scotoma, headache (45), amnesia, and auditory agnosia (106), motor aphasia, slight facial paralysis, a positive Babinski in one leg, agraphia, and a stuporous condition (22, 59, 136). Other temporary effects of decompression to altitude on the central nervous system are changes in muscle tone and in the strength of a variety of reflexes, and localized sensory losses. The incidence of *temporary* dysfunction of the central nervous system was given as 0.28 per cent, of a large series of exposures at 34,000 feet with oxygen (22). Temporary damage of the central nervous system may be even rarer than indicated, because of the difficulty of ruling out simulated effects caused basically by emotional disturbances. The only recorded instance of uncomplicated *permanent* damage to the central nervous system was reported by Smith (149) who observed paralysis in the hind limb of a dog soon after explosive decompression to 45,000 feet.

Briefly summarized, it appears that: 1. Neurologic signs of damage to the central nervous system following decompression to altitude are rare, when compared with the consequences of aeroembolism from deep diving. This relative infrequency of lesions in the central nervous system must be attributed to the relatively smaller amount of gas in solution in the body at altitude. 2. With the single exception noted above, neural damage is temporary. This may be attributed to the reduced volume of gas present in the body at altitude and to the differential between gas bubbles assumed to exist in relation to the affected regions and the high oxygen content of inspired gases. This procedure of reducing the effects of aeroembolism has been recommended for caisson workers ever since the time of Paul Bert, and is in common practice at the present time (11). 3. The neural symptoms are non-specific. As in the case of diving, this property may be attributed to the factor of chance of where a given gas-bubble freely circulating in the blood will finally lodge, occlude the local circulation and cause temporary dysfunction.

Further complicating the picture is the possibility that gas bubbles may lodge in intraneural blood vessels, and thus cause local neural (at least temporary) damage. Gas bubbles were observed in such sites in a study of acute, fatal aeroembolism in rabbits (51). An exhaustive search for gas bubbles in rabbits decompressed to altitude was made by Trowell (160). His failure to find these may be due to the inadequacy of the method of fixation employed.

Lung. Pressure. As a consequence of aeroembolism gas bubbles may occur in the pulmonary artery and its branches and in the chambers of the right side of the heart in sufficient numbers to cause death through occlusion of the circulation and asphyxia. In order to achieve asphyxia, it is necessary for enough blood vessels to be occluded to reduce the pulmonary arterial blood flow by 52–66 per

cent (64). In addition there may be pulmonary hemorrhage, edema, emphysema, and atelectasis (18, 21, 46, 80, 82). Except for minor details, the mechanisms of injury were developed by the early workers in the field. Gas bubbles arising in venous channels pass through the right auricle and ventricle to the pulmonary artery. The emboli occlude larger and smaller branches, but, contrary to earlier assertions by others, never reach the capillary bed; they are sometimes present in bronchiolar vessels (51, 56). The smallest vessels occluded were 40 μ in diameter. This corresponds with the direct observations of Chase (30) on the mesenteric vessels of living animals that gas bubbles introduced into the arterial circulation never penetrate beyond arterioles of a similar diameter. The occlusion of arterioles of similar dimensions was reported in other sites also by Curtillet (36) and by Tureen and Devine (162). The occluded blood vessels may be markedly stretched at the site of the gas bubble, as well as centrally. It should be noted in passing that the lung differs from all other organs in that gas bubbles in this structure represent emboli, with no significant contribution of local origin. While nearly all gas emboli are retained in the lung, it has been believed from the earliest writers (18) that some pass through the organ to be distributed as arterial emboli by routes which are largely hypothetical: 1, intrapulmonic arterio-venous anastomoses (36), and 2, the blood "lakes" described by Sjöstrand (147).

The relation of experimentally induced gas emboli in animals to aeroembolism appears to have been most extensively investigated by Heller, Mager and v. Schrötter (80). They found that the distribution of gas bubbles in both instances was strikingly similar. In the case of air introduced intravenously, the final distribution depended on the volume of gas injected, the rate of injection and the size of the gas bubbles.

Pulmonary hemorrhage, edema and emphysema have been shown to follow pulmonary embolization by solids (41, 165). Hemorrhage and emphysema have been attributed also to the violent rupture of alveolar walls (82). Pulmonary atelectasis as a result of decompression was first described by Bert, who attributed it to possible rupture of a vesicle at the time of decompression, thus permitting access of air under pressure to the pleural space. Other possible mechanisms are: 1, overinflation of the lung while inflating the Eustachian tube during compression; 2, expansion of air in alveoli and rupture of their walls, as a consequence of holding the breath (26); 3, escape of gas under pressure from alveoli to interstitial tissue, along the vascular sheaths to the hilus of the lung, pneumo-mediastinum, and pneumothorax (107, 108); and 4, rupture of a few alveoli into the retropleural connective tissue in the manner of a one-way valve, escape of this gas retropleurally to the hilus, pneumomediastinum, and pneumothorax (55). The mechanisms enumerated are applicable not only to the rare instances observed after decompression (4, 130, 131), but also to other equally rare situations: spontaneously (67, 120), in severe straining activity (111) and in cases of obstructed air passages, such as accompany diphtheritic laryngitis (39), following pertussis or other infections (31, 96, 111).

Altitude. Hoppe-Seyler in 1857 (84) attributed death to occlusion of pulmo-

nary capillaries by gas bubbles. The few other observations on this phenomenon were contradictory. They showed that rather few bubbles appeared in branches of the pulmonary artery, while only one bronchiolar vessel was found to be filled with gas in the series of six rabbits studied. The small number of pulmonary bubbles as compared with those in animals decompressed from pressure is explainable by the smaller quantity of gas in the body of animals decompressed to altitude. The same observation has been cited also as one of several reasons for the belief that animals decompressed to altitude tend to die of embolism of the central nervous system, rather than of pulmonary asphyxia, a condition opposite to that believed to obtain in animals decompressed from high pressure atmospheres (27, 56).

Pulmonary hemorrhage occurred irregularly in rabbits decompressed to altitude (27). Smith (149), and Berg, Baumberger, et al. (16), found that it follows more regularly after explosive decompression of experimental animals, especially, as the latter found, when decompression took place during inspiration or the last two-thirds of expiration. Only two instances of mediastinal emphysema have been reported in men after explosive decompression (112). Pneumothorax, retroperitoneal emphysema, and pulmonary atelectasis also occurred in rabbits, especially after slow decompressions. The administration of nembutal or of carbon-dioxide mixtures predisposed to pneumothorax, while preoxygenation or the administration of ammonium chloride, lactic acid, or sodium bicarbonate did not affect the incidence of atelectasis (28). The mechanism has not been investigated.

The observations on rabbits confirmed in part earlier reports by a group of British investigators who described the occurrence of atelectasis, vascular congestion, and pneumothorax in rabbits, rats, and guinea pigs decompressed to altitude (49, 50, 77, 78, 79, 159). They showed that these changes were related to the final altitude, and were unaffected by the degree of anoxia or by the CO_2 content of the blood. In unpublished experiments, the writers found that atelectasis was more common in young than in older animals.

Blood vessels. Ever since 1857 (Hoppe-Seyler (84)) it has been known that bubbles are more commonly (sometimes exclusively) present in the large veins following decompression from high pressure atmospheres, or to altitude. This was attributed by Bert to the fact that venous pressure is lower than that in arteries. Harvey et al. (72) added another factor, namely, the higher CO_2 tension of venous blood, facilitating the growth of gas bubbles. Arterial gas bubbles, when present, were attributed by Bert to passage of some gas bubbles through the lung into the left side of the heart and their distribution as emboli. Under extreme conditions, where the effect of blood pressure becomes overshadowed by other factors, one would expect gas bubbles to arise in arteries in the same manner as in veins. The distribution of gas bubbles is in fact similar to that which has been described repeatedly after the rapid intravenous injection of large volumes of air. Smaller volumes of air (or air injected at a slow rate) produce bubbles that are confined largely or almost entirely to the venous system. After decompression from high pressure atmospheres, gas is sometimes present

in the capillary bed of several tissues (52), but this seldom occurs, and then in fat tissue only, after decompression to altitude (51). The difference must be attributed to the vastly greater amount of gas in the tissues in the first condition.

Recently it has been noted by direct observations of living animals that an arteriolar and capillary constriction may occur at altitude. This has been observed to take place in man, rats and frogs (99, 104, 121), though not in cats (125). Reed and Blinks (138) found that vasoconstriction was not related to bubble formation. They also found that it could be abolished by nerve section and by exercise. It was correlated in man with the prolonged refilling time of the finger nail bed and of the arm vein (99), and with reduced skin temperature (157). This group found evidence that the latter phenomenon is related to susceptibility to bends.

Knisely (99) failed to see evidence of intravascular sludge in men exposed to altitude, although he did describe vasoconstriction. On the other hand Patek, who failed to note any change in the caliber of blood vessels in cats, observed sludging (125). This may have been due to the anoxic condition of the animals.

Sludging was observed directly in the pial vessels of living animals injected intravenously with air or decompressed from high pressure atmospheres. It occurred after vascular occlusion by gas bubbles, presumably as a consequence of reduced circulation and anoxia (170, 172). End (44) regarded agglutination of red blood corpuscles as the causative factor of decompression sickness, and gas bubbles as a secondary event. Swindle et al. (154) attributed disturbed function of decompression sickness to the occurrence of fragile, non-gaseous plasma flocculates. Jacobs and Stewart (88) investigated these possibilities by studying rat blood exposed to high pressure atmospheres and then decompressed. They found no clear evidence of any change in sedimentation rate, tendency to rouleaux formation or in the aggregates of rouleaux. They failed to observe true agglutination of red blood cells, but did describe a tendency for blood platelets to form aggregates about small gas bubbles and to appear free in this form later. Such platelet aggregates could conceivably lead to occlusion of small vessels in certain regions of the body. Gersh and his co-workers paid particular attention to the possible occurrence of all of these phenomena and failed to detect them in animals decompressed from high pressure atmospheres or to altitude. As the method employed (fixation by freezing and drying) could be confidently expected to preserve such flocculates and aggregates, the failure to find them returns the causation of decompression sickness to the conventional realm of gas bubbles.

Fat Tissue. Pressure. Experience in diving or caisson operations is that accidents occur more commonly in fat workers than in lean. Heller, Mager and v. Schrötter (80) reported that animals rich in fat in the subcutaneous tissues, mesentery and pericardium are more prone to show abnormalities than lean animals. This was tested for the first time by Boycott and Damant (20), when they found that the time of survival of rats and guinea pigs after decompression from a stated pressure was related to their total fat content. This was confirmed in another way by observing the highest pressure which guinea pigs could survive when decompressed rapidly, and relating it to their total fat content. For ex-

ample, fat guinea pigs (average fat content 31.9 per cent) survive decompression from a pressure of 45 lbs/sq. in., but die when brought up from 60 lbs/sq. in.; on the other hand, lean guinea pigs (average fat content 8.2 per cent) usually survive decompression from an atmosphere at 90 lbs/sq. in. but die when brought up from 105 lbs/sq. in. (52). Using another criterion (i.e., the time of appearance of the first gas bubble), the tendency for bubble formation in resting cats was also found to be related to the fat content of the animal when it was decompressed from moderately increased pressures. But in active animals, or at greater pressures, this relationship was masked by other factors (70).

Fat tissue containing numerous gas bubbles appears foamy, like whisked white of egg (82). The bubbles may be intravascular as well as extravascular (20). As a consequence, in surviving animals, large masses of necrotic fat may be present, especially below the kidneys. In late stages, the necrotic regions are surrounded by a zone of giant-cells, with some of the fat converted to calcium soap (21). In guinea pigs, minute gas bubbles may occur in the intracellular fat inclusions of fat cells. In somewhat more severely affected animals, macroscopic bubbles which are extravascular and extracellular may occur. Intravascular gas bubbles may be present in fat tissue in the absence of the aforementioned bubbles. Hemorrhage and vascular distention are associated frequently with the occurrence of extravascular gas, especially in fat animals (52). Thus, it may be assumed that, when purpuric subcutaneous spots appear in divers, gas bubbles have occluded some blood vessels and vascular congestion and hemorrhage have taken place.

The genesis of gas bubbles in fat tissue may be reconstructed in some such manner as follows: Fat cells dissolve excess amounts of gas, especially nitrogen, during pressurization. During and after decompression, many fat cells enlarge due to the appearance in the fat inclusions of minute intracellular bubbles. Meanwhile dissolved gas passes from the fat inclusions to the tissue fluid and the circulating blood. Depending on a number of factors, gas continues in solution or forms visible circulating bubbles. When these exceed the diameter of the blood vessels, they occlude the circulation. Meanwhile, in some regions, the cells increase in volume more markedly due to the increased number of intracellular gas bubbles. These distended cells then rupture and discharge their contents into an irregularly outlined intercellular (extravascular) bubble which contains cellular debris, fat, and gas under pressure. About this time, some blood vessels occluded by gas bubbles are distended with blood, and others may rupture.

This hypothetical reconstruction of the events leading to the formation of gas bubbles in fat tissue is supported by evidence along at least three lines that certain physical factors are involved. These are as follows: 1. The increased solubility of gases in fat as compared with tissue fluid (see p. 368). Biological evidence may be cited. The occurrence of gas bubbles in fat tissue and their number are directly related to its lipid content. Extravascular gas bubbles occur only in lipid rich structures (fat tissue, adrenal cortex, myelin sheaths of peripheral nerves) (52). Finally, gases which are especially soluble in lipid (such as argon),

are much more effective in producing gas bubbles in these structures, while gases which have a low $\frac{\text{fat}}{\text{water}}$ index of solubility are relatively ineffective (53). 2. The increased amount of gas in solution in fat is directly related to the number of gas bubbles formed in fat tissue. This was illustrated by the greater severity of the effects of decompression when animals are pressurized at higher pressures. It was also shown by a comparison of the effects of argon-oxygen, and nitrogen-oxygen mixtures. Although the relative solubilities in lipid and tissue fluid of argon and nitrogen are nearly identical, the amount of the former in solution is over twice as great. The number of gas bubbles appearing in fat tissue is far greater when argon is used than when nitrogen is used. In both examples, variations in the number of gas bubbles occur although the size of the gas bubbles remains the same. The size of the gas bubbles is probably referable to some tissue property such as elasticity. 3. The poor capillary density of fat tissue is an important factor predisposing to bubble formation, since excess gas cannot be removed rapidly enough to avoid high ΔP values in the tissue (see p. 362).

Altitude. In contrast with the serious consequences of excess body fat for divers, fat is not of great significance in bubble formation on decompression to altitude. Harvey et al. (70) and Catchpole and Gersh (27) failed to find any correlation between the fat content of animals decompressed to altitude and the time of first appearance of intravascular gas bubbles, the severity of the symptoms, or the time of survival.

Extravascular gas bubbles do not occur. Gas bubbles have been observed in veins draining fat depots but less commonly than in blood vessels draining muscles (70). They were found to be somewhat more numerous in fat tissue than in others by Gersh and Catchpole (51). Fat is the only tissue where gas bubbles were present in the capillary bed.

Muscle. Pressure. Little emphasis is placed in the literature on muscular lesions following decompression except as a consequence of neurological damage, or in the case of regional infarct (169). Only in recent years has muscle acquired great significance in theories on the origin of gas bubbles. Bert (18) found bubbles to be present only in intermuscular fascia, and Boycott et al. (21) were unable to see gas bubbles in skeletal muscle. Harvey et al. (70) found that in anesthetized cats bubbles do not appear in veins draining largely muscular regions, although they are present in vessels from fat depots. On the other hand, in stimulated animals, bubbles arise only from large muscle masses. Both the Princeton and the California groups agree that violent activity increases the number of bubbles (70, 177). In normal, decompressed guinea pigs, gas bubbles were present in muscle in arteries, capillaries, and veins of all dimensions, but they were fewer in number than in blood vessels of fat depots (52). Under the same conditions, an equally small number of blood vessels were occluded after decompression from a helium-oxygen mixture, a larger number were observed after argon, and none were observed after oxygen.

Muscle is regarded as an important site of bubble formation, the gas phase appearing in regions of mechanical tension, whether this is induced by passive

movement, stretching, injury or stimulation. This is attributed to the decreased P obtaining near tendon attachments due to the local stresses (68, 110). It should be stated that in guinea pigs, although blood vessels near the knee joint were not noted for their density of gas bubbles, those accompanying the *long* tendons near the ankle joint displayed gas bubbles prominently (56). Also important are the increased volumes of gas in solution as a result of compression. The increased gas content appears to overshadow the facilitating action of carbon dioxide.

Altitude. In anesthetized, decompressed animals, relatively few gas bubbles appear, and these seem to originate in fat depots. In stimulated limbs, however, the numerous bubbles appear to arise primarily from muscle (27, 28, 69, 110). By direct visualization, no bubbles were found in muscles of decompressed rats, although they were present at autopsy in the vena cava and other large veins (104). Arterioles were observed to be constricted. On the other hand, small numbers of bubbles were described in decompressed rabbits in blood vessels of larger-than-capillary dimensions. The factors responsible for bubble formation are primarily: 1, local production of CO_2 in regions of muscular activity, so that gas nuclei or minute bubbles in regions of high CO_2-tension tend to expand rapidly; 2, mechanical tension induced by passive movement, stretching, injury or electrical stimulation, which results in regions of low pressure in the neighborhood of insertions (70, 72, 110, 177, 179).

Bone Marrow, Bone, Periosteum, Joints. Bone marrow. The occurrence of gas bubbles in bone marrow has been described only in acute preparations of guinea pigs decompressed from compressed air (56). It was rather unexpected to find that in yellow marrow which was equivalent to lipid-rich fat, intravascular bubbles were exceedingly rare and extravascular bubbles were absent while the fat of the same animals was filled with numerous bubbles in both sites. Only under more severe conditions of pressurization did extravascular gas bubbles appear, together with a larger number in arteries, sinusoids, and veins. The extravascular gas bubbles were frequently larger than in fat, more irregular, and with a tendency to be concentrated near bony spicules. Sometimes, they seemed to dissect the endosteum from the marrow tissue. Arterial occlusion by gas is probably the result of embolism. Venous and sinusoidal gas bubbles probably arise locally in the bone marrow due to the slow circulation (24), the increased amount of nitrogen stored in the inclusions of the fat cells, and the low blood pressure in the blood channels. The extravascular distorting bubbles probably arise in a manner similar to that described for fat tissue. Both the large size of the extravascular bubbles and their irregular distribution may be due to the intermittent flow of blood through some sinusoids. This would result in a high uptake of excess gas in some regions and a large t locally, with bubble formation, and in other regions, low uptake of gas and no bubble formation. The tendency for more bubbles to occur in relation to bony spicules and to dissect the endosteum may be related to the finding that some crystals tend to favor the origin of gas bubbles from gas nuclei (126). It should be emphasized again that gas bubbles in bone marrow are relatively scarce, and then even so they

occur only in extreme conditions. This is probably due to the retarded rate of saturation of bone marrow with excess gas and the reduced circulation.

Bone and periosteum. Pressure. Bone necrosis as a result of decompression sickness was first noted in 1888 (163). The literature on the subject has been thoroughly reviewed by Kahlstrom, Burton and Phemister (95). Additional information has been supplied by Phemister (127), Coley and Moore (32), and Rendich and Harrington (139). The most detailed microscopic study was made by Kahlstrom et al. They found that when necrotic bone was situated in the epiphysis and bordered on joints, there were varying degrees of collapse of the weight-bearing portions, and invasion of the necrotic regions with new bone or calcification. The overlying articular cartilaginous surfaces were replaced by fibro-cartilage, resulting in arthritis deformans. When the necrotic bone was located in the diaphysis, or in the epiphysis removed from articular surfaces, replacement by new bone or calcification also took place, but collapse and deformation did not occur. Since these observations were made several years after the original trauma, the mechanism could not be determined exactly. They postulated two general methods by which gas bubbles could cause bone atrophy: 1, gas bubbles form in bone marrow and interrupt the circulation of bone, and 2, gas emboli in numbers of smaller branches of the nutrient artery occlude the osseous circulation. Both mechanisms were demonstrated in work on decompressed guinea pigs. In some instances, gas-filled Haversian vessels were observed (56). It should be pointed out that bone changes in caisson workers and divers are relatively uncommon, probably for the same reasons that were given to account for the scarcity of gas bubbles in bone marrow. Gas bubbles are even scarcer in the periosteum, where, only rarely, gas distended small blood vessels were seen (56).

Altitude. Only one systematic effort has been made to detect bone damage in men decompressed to altitude. No radiographic evidence of such effects was found in a rather large number of subjects with 5–100 hours' exposure at 35,000 feet or higher. Follow-up x-rays several years later were recommended (135). The probability is that bone damage will be exceptional, for the same reasons that neural damage in aviators is scarcer than in divers (see p. 375). Another solitary report on the appearance of periosteum in living animals failed to disclose any evidence of bubbles in this site (125). However, the altitude at which the animals were studied, and the rate of decompression may have been unfavorable.

Joints and periarticular structures. Pressure. Experimental studies utilized morphological methods for the demonstration of gas bubbles or their effects, and x-rays to locate gas bubbles photographically. In guinea pigs decompressed from high pressure atmospheres, gas bubbles may be present in the joint fat. But even when they are absent, blood vessels in the region, especially those adjacent to the long tendons are frequently occluded and distended by gas bubbles (56). With x-rays, only the larger blood vessels which were completely filled with gas, were recognizable. Bubbles in fat or connective tissue were indistinguishable, or when they were very numerous, were recognizable almost always as a general x-ray shadow. In only one instance, in which shadows suggestive of

bubbles were found, only a minute fraction of all the bubbles were recognizable as such, because of the small size of the bubbles, poor resolution and overlay (57).

Altitude. The only morphological studies reported were on the joints of living animals. The failure to find gas bubbles in the periarticular tissues may have been due, however, to experimental conditions (125). Webb and co-workers (174) attempted to correlate gas in tissues of the knee with pain, and found that pain is unrelated to gas in the joint space or in the suprapatellar bursa. Better statistical relationships of pain were found with x-ray photographs correlating, in decreasing order of success, with 1, streaks in tissues posterior to the joint capsule; 2, gas bubbles immediately posterior to the joint space, and 3, gas bubbles in the infrapatellar space. The streaks appeared to be extravascular collections of gas in the intermuscular fascial planes of the posterior tissues of the leg. The gas seemed to be disposed as a collection of minute bubbles and to have no relation to the *larger* blood vessels or nerves of the region. Similar findings were reported by Thomas and Williams (155), who pointed out that "streaking" may occur in the absence of pain, and that there was no characteristic x-ray picture with which pain is correlated. Lund et al. (105, 106) came to the same conclusion in their study on the origin of pain in bends, and expressed the belief that the demonstration of gas by radiographic measures alone can be expected to help very little in supporting theories on the intimate origin of pain. A similar attitude was expressed by Gersh (57), who pointed out the difficulties in interpreting x-ray photographs of gas bubbles in terms of finer anatomical structures.

Adrenal Gland. Pressure. Gas bubbles have been detected in all layers of the adrenal cortex, as well as in the medulla. In addition, they have been observed in the capsular arteries, in the medullary veins and its tributaries, and occasionally in the reticular sinusoids. In all layers of the cortex, but most frequently in the fascicular zone, gas bubbles are present also extravascularly. Three major pathological consequences are 1, the occurrence of hemorrhages in the vicinity of the gas bubbles; 2, rupture of cortical cells in the region, and 3, vascular congestion in the sinusoids peripheral to the gas bubbles, with cessation or reduction of blood flow (52).

Two main factors are important in the origin of gas bubbles in the adrenal gland: the high content of lipid and certain peculiarities of the vascular pattern. The following sequence of events may be reconstructed: Gas bubbles begin as small occluding emboli in capsular arterioles and as clusters in the sinusoids of the reticular zone after the blood has passed through the fat- (and nitrogen) rich fascicular zone. These may grow and further occlude the circulation in the cortex. These areas of reduced cortical blood flow are small, and surrounding regions may have a normal circulation. With the two regions having different gas tensions, the gas bubbles continue to grow in the former region, and destroy the cortical organization, particularly in the fascicular zone, where the fat and dissolved gas is richest. The general disruption of structure is accompanied by hemorrhage, and also by a further sinusoidal congestion. Meanwhile, in the adjacent unaffected region, gas in solution is removed by the circulation either in solution or as minute bubbles which do not clog the sinusoids. Gas bubbles

arising in this manner may, in the capacious venous channels of the medulla, where the blood pressure may be smaller, expand to form large intravascular gas bubbles which may seriously interfere with the circulation throughout the gland.

Although gas bubbles appear in the adrenal gland of decompressed guinea pigs, it should be emphasized that they form somewhat less readily than in fat tissue. It is difficult to evaluate the clinical significance of the pathological findings. The temporary withdrawal of functioning cortical tissue may be related to the sense of exhaustion which divers frequently report after immersion.

Altitude. Gas bubbles were observed only in capsular arterioles and in some of the large venous medullary channels in rabbits; none were found in sinusoids or extravascularly (51).

Kidney. Pressure. Destruction of renal tubules of the cat was prominently figured by Boycott, Damant and Haldane (21). The proximal convoluted tubules of the cat are virtually unique because they are exceedingly rich in lipid, and it is unlikely that similar effects will occur in the kidney of other animals. In support of this assertion is the rapidity of the blood flow, and the brief desaturation time of renal tissue (see p. 370).

Altitude. Gas bubbles were present only in renal blood vessels of large caliber in rabbits decompressed rapidly (51).

Liver. Pressure. Boycott, Damant and Haldane (21) found numerous bubbles issuing as a froth from the cut surface of the liver of decompressed animals. These were believed to be confined to blood vessels. With methods less subject to artefact, very few microscopic gas bubbles were seen infrequently and only in central veins (52). Gas bubbles were common after decompression from a high pressure atmosphere of argon-oxygen (when some bubbles were observed in sinusoids), were rare after helium-oxygen mixtures, and were absent after oxygen (53).

A peculiar finding was the appearance of watery vacuoles in the cytoplasm of liver cells. The spherical vacuoles displace and distort the nucleus, and contain no fat stainable with Sudan III. They were present in liver cells not only after decompression from compressed air, but also from atmospheres of argon-oxygen, helium-oxygen, and oxygen. Their significance is unknown (52, 53).

The scarcity of bubbles in the liver may be explained by the work of Campbell and Hill (25) who found that liver has a rather slow half-saturation time. This may be due to the fact that most of the hepatic blood has already passed through a capillary bed and given up most of the excess gas. In addition to the reduction of the gas volume in the liver due to this factor, the nature of the arterial supply may contribute to the total picture during and after decompression. The less saturated arterial blood emptying into the sinusoids would tend to reduce the gas tension of blood in them and in the central vein and thus reduce the tendency for bubble formation.

Altitude. In decompressed rabbits, microscopic bubbles were observed very seldom, in the central vein and in the branches of the portal vein. The intracellular vacuoles observed in animals decompressed from high pressure atmospheres did not appear. They have, however, been described in men who died of the effects of exposure to altitude and in animals subjected to sudden reduction in

atmospheric pressure (103, 158). Ladewig gave good evidence to indicate that they may be related to low oxygen tension and anoxia rather than the effects of decompression. On the other hand, it is difficult to see how anoxia would play a rôle in the appearance of the vacuoles of guinea pigs decompressed rapidly enough from high pressure atmospheres to result in death in one to three minutes.

Spleen. Pressure. Gas bubbles occur in splenic sinusoids, arteries, and veins, in decreasing order of frequency, of guinea pigs decompressed rapidly from high air pressures (56). The sinusoidal bubbles are probably related to the intermittency of blood flow and possibly the reduced rate of blood flow in this structure. Both factors may result locally in regions of high gas tension (in closed sinusoids or in those with sluggish circulation) adjacent to regions of low gas tension (in narrow sinusoids with rapid circulation). If the volume of gas in the adjacent tissue is large enough, the gas bubbles grow and cause large, tearing defects to appear. The arterial bubbles are probably the result of gas emboli. The venous bubbles probably originate in situ because of the increased gas tension and decreased blood pressure.

Altitude. Fewer gas bubbles were present in the sinusoids of rabbits decompressed to altitude; they were present also in arteries and veins. In two rabbits, large, subcapsular, tearing bubbles were visible (51). The factors responsible for their appearance are probably the same as those described above.

Site of Origin of the Bends and the Chokes. Although much has been written on the subject, the literature dealing with unequivocal, direct evidence is distressingly scanty. If one makes the logical assumption that the pain of the bends and the chokes is due to gas bubbles, then from the point of view of the pathologist, those regions which are prone to bubble formation in man or other animals under less extreme conditions of decompression acquire the greatest significance as possible sites of the origin of pain. Whether pain results from distortion of nerve endings or nerve fibers by intra- or extravascular gas bubbles, or from local ischemia arising from reduced or occluded circulation is unknown. Gas bubbles have been described in muscle, intermuscular fascia, fat tissue, nerves, bone marrow, periosteum, and in fascial tissues adjacent to joints or to the long tendons. Except for fat tissue (where intracellular bubbles may occur in the cytoplasmic fat inclusion and also extravascularly) and for the myelin sheath of nerve fibers, no other extravascular gas bubbles have been noted in organs of locomotion. Even in these sites, gas bubbles may be seen in blood vessels in animals decompressed from high pressure atmospheres before they appear extravascularly; in animals decompressed to altitude, only intravascular bubbles occur. In all other regions, gas bubbles are confined entirely to the blood vessels. Thus, granting the primary assumption, the conclusion seems inescapable that the site of origin of gas bubbles is primarily vascular, though it cannot be denied that the extravascular gas bubbles may in addition cause pain by distortion. The evidence is cited in previous sections for the conclusion that the blood vessels most likely to be involved in the causation of the bends are those in the vicinity of the joints or tendons near their origin or insertion. However, others located in

nerves, muscles, or fasciae (including periosteum) may also be additional sites where pain may originate. A similar analysis of the anatomic basis of the pain of the chokes leads to the conclusion that the most likely sites of origin are the branches of the pulmonary arteries.

The conclusions derived above must be modified to a certain extent in the light of evidence showing that vascular occlusion by a gas bubble is accompanied by vascular congestion (30). If pain arises from vascular distention, then the same blood vessel or branches thereof somewhat removed from the gas bubble may be stretched to nearly the same extent. If pain arises from ischemia, then any point in the anoxic region may result in stimulation of the proper nerve endings or fibers.

Effects of Exercise and Drugs on Aeroembolism. Pressure. The conflicting claims of the effects of exercise on divers may be resolved by a critical analysis of studies by Harris et al. (68) and by Harvey et al. (70). The first group found that the minimal pressure which results in the liberation of bubbles after decompression is markedly lowered as a result of exercise. It may be assumed that as the pressure is increased greatly over this minimum value, the amount of gas present in the body outweighs in importance the decreased P which accompanies muscular activity. The result of this is that it would be difficult in practice to show that there is any relationship between exercise and amount of gas bubbles formed on decompression from higher pressures.

The effects of exercise were illustrated in another way by the use of anesthetics. Anesthetized animals, probably as a result of decreased muscular tone, had a much higher minimum effective pressure. Harvey and his colleagues (70) made similar observations, and found that a clear relationship between exercise and bubble formation existed on decompression from lower pressures, but that this is masked after higher pressures. From these results it is possible to understand that in divers, where the time under pressure, the pressure, and the rate of decompression are not often duplicated in experiments, some workers may claim that exercise is harmful, ineffective, or even beneficial in preventing or ameliorating the bends. Harris et al. (68) also reported that the use of anesthetics reduced bubble formation markedly, probably as a result of decreased muscular tone.

Oxygen administration is used effectively for the prevention and treatment of the more serious effects of aeroembolism in divers (5, 11, 89). This is confirmed by experiments on guinea pigs, in which far fewer gas bubbles in all sites were noted after decompression from oxygen as compared with nitrogen (53). This is difficult to understand, as the solubility properties of both gases are very nearly identical. Certain phases of decreased aeroembolism may be explained by assuming that oxygen of gas emboli is used metabolically, thus, in effect, reducing t at the site of bubble formation.

Altitude. The accelerating effects of exercise on bubble formation in animals at altitude have already been described. They correspond with the well-known increased severity of symptoms induced in men decompressed to altitude under controlled conditions. The increased tendency to bubble formation is attributed

to decreased P in the region of tendon attachments, and increased local t values due to excessive accumulation of CO_2

Preoxygenation is effective in delaying the onset of symptoms of aeroembolism in men, and in protecting animals against bubble formation (see p. 369). This effect is achieved presumably by substituting a metabolizable gas (reducing t) for nitrogen, which is largely eliminated during the period of preoxygenation.

The administration of ammonium chloride and lactic acid was also found to reduce the tendency to bubble formation in decompressed rabbits (28). In man (2, 3, 113) ammonium chloride increases tolerance to anoxia and improves subjective symptoms. The underlying mechanism in the latter studies appear to be a shift in the blood pH towards the acid side and correction of the alkalosis caused by hyperventilation and loss of CO_2. To the extent to which CO_2 contributes to the initiation of bubble formation at altitude, a lowering of the arterial CO_2 tension would be expected to hinder bubble formation. The administration of moderate concentrations of CO_2 or of sodium bicarbonate did not have a noticeable effect on bubble formation (28, 110, 177). Accordingly, it was not surprising that in men exposed for two hours at a simulated altitude of 38,000 feet, the symptoms were of the same order whether the subject breathed pure oxygen or oxygen diluted by CO_2 (60).

There has been no uniform interpretation of the results of administering vasodilator drugs. Aminophyllin has been claimed to alleviate, or to reduce the severity of the bends (180); on the other hand, it had no effect on bubble formation in animals (138). A favorable effect of d-amphetamine in reducing the incidence of incapacitating bends has been both proposed (87) and denied (141).

SUMMARY

The evidence is overwhelming that gas bubbles are the primary pathogenetic agent in eliciting the pathologic effects of decompression sickness. Whether they occur after decompression from high pressure atmospheres or to altitude, gas bubbles are chiefly intravascular, and they are held to be responsible for nearly all important phases of the syndrome of decompression sickness. Extravascular gas bubbles occur also under certain severe instances of decompression from high pressure atmospheres, but they are restricted to certain lipid-rich structures. The pathological effects may be vastly greater after decompression from high pressure atmospheres than to altitude. These are described in detail for the various tissues and organs of the body. An attempt has been made to relate earlier and recent findings to each other, and to the causative factors. Basic to an understanding of the mechanisms involved in the syndrome is a consideration of the physical factors responsible for the uptake and elimination of excess gas in the body. The following physical factors were subjected to analysis: intrinsic factors such as blood pressure, blood flow, tissue permeability, tissue activity, the chemical composition of tissues and the solubility of gases in body components, and extrinsic factors such as the type, rate and extent of decompression applied.

REFERENCES[1]

(1) Armstrong, H. G. Principles and practice of aviation medicine. Williams & Wilkins Co., Baltimore, 1939.

(2) Barach, A. L., W. L. Bloom, M. Eckman, C. Rule and C. C. Rumsey. The acid-base balance of the blood following ingestion of ammonium chloride. Committee on Aviation Medicine, Report No. 240, National Research Council, December 24, 1943.

(3) Barach, A. L., et al.; R. Hodes and M. G. Larrabee. Summary of reports on the effect of ammonium chloride on altitude tolerance. Committee on Aviation Medicine, Report No. 96, National Research Council, December 1942.

(4) Behnke, A. R. Analysis of accidents occurring in training with the submarine "lung". U. S. Nav. Med. Bull. **30:** 177, 1932.

(5) Behnke, A. R. The application of measurements of nitrogen elimination to the problem of decompressing divers. U. S. Nav. Med. Bull. **35:** 219, 1937.

(6) Behnke, A. R., Jr. Physiologic studies pertaining to deep sea diving and aviation, especially in relation to the fat content and composition of the body. The Harvey Lectures, 198, 1941–1942.

(7) Behnke, A. R. The absorption and elimination of gases of the body in relation to its fat and water content. Medicine **24:** 359, 1945.

(8) Behnke, A. R. Decompression sickness incident to deep sea diving and high altitude ascent. Medicine **24:** 381, 1945.

(9) Behnke, A. R. The absorption and elimination of gases of the body in relation to its fat and water content. Medicine **24:** 359, 1945.

(10) Behnke, A. R., Jr., B. G. Feen and W. C. Welham. The specific gravity of healthy men. J. A. M. A. **118:** 495, 1942.

(11) Behnke, A. R., L. A. Shaw, A. C. Messer, R. M. Thomson and E. P. Motley. The circulatory and respiratory disturbances of acute compressed-air illness and the administration of oxygen as a therepeutic measure. Am. J. Physiol. **114:** 526, 1936.

(12) Behnke, A. R., R. M. Thomson and L. A. Shaw. The rate of elimination of dissolved nitrogen in man in relation to the fat and water content of the body. Am. J. Physiol. **114:** 137, 1935.

(13) Behnke, A. R. and T. L. Willmon. Gaseous nitrogen and helium elimination from the body during rest and exercise. Am. J. Physiol. **131:** 619, 1940.

(14) Behnke, A. R. and T. L. Willmon. Cutaneous diffusion of helium in relation to peripheral blood flow and the absorption of atmospheric nitrogen through the skin. Am. J. Physiol. **131:** 627, 1940.

(15) Behnke, A. R. and O. D. Yarbrough. Physiologic studies of helium. U. S. Nav. Med. Bull. **36:** 542, 1938.

(16) Berg, W. E., J. P. Baumberger, F. Crescitelli, S. Rapaport and P. O. Greeley. Explosive decompression: lung damage correlated with the respiratory cycle in explosive decompression. Committee on Aviation Medicine, Report no. 173, National Research Council, August 16, 1943.

(17) Berg, W. E., M. Harris, D. M. Whitaker and V. C. Twitty. Additional mechanisms for the origin of bubbles in animals decompressed to simulated altitudes. J. Gen. Physiol. **28:** 253, 1945.

(18) Bert, P. La pression barométrique; recherches de physiologie experimentale. Paris, G. Masson, 1878.—Barometric pressure. Transl. by M. A. Hitchcock and F. A. Hitchcock, Columbus, Ohio, College Book Co., 1943.

(19) Blanchard and Regnard. Cited by Heller, Mager and v. Schrötter, 1881.

(20) Boycott, A. E. and G. C. C. Damant. Experiments on the influence of fatness on susceptibility to caisson disease. J. Hyg. **8:** 445, 1908.

(21) Boycott, A. E., G. C. C. Damant and J. S. Haldane. The prevention of compressed-air illness. J. Hyg. **8:** 342, 1908.

[1] To June, 1946.

(22) Brown, G. A., C. H. Cronick, H. L. Motley, E. J. Kocour and W. O. Klingman. Nervous system dysfunction in adaptation to high altitude and postflight reactions. War Med. **7:** 157, 1945.

(23) Campbell, A. and L. Hill. Concerning the amount of gas in the tissues and its removal by breathing almost pure oxygen. J. Physiol. **71:** 309, 1931.

(24) Campbell, A. and L. Hill. Studies in saturation of the tissues with gaseous nitrogen. I. Rate of saturation of goats' bone-marrow in vivo with nitrogen during exposure to increased atmospheric pressure. Quart. J. Exper. Physiol. **23:** 197, 1933.

(25) Campbell, J. A. and L. Hill. Studies in saturation of tissues with gaseous nitrogen. III. Rôle of saturation of goat's brain, liver and bone marrow, in vivo, with excess nitrogen during exposure to +3, +4, and +5 atmospheres pressure. Quart. J. Exper. Physiol. **23:** 219, 1933.

(26) Case, E. M. and J. B. S. Haldane. Human physiology under high pressure. I. Effects of nitrogen, carbon dioxide, and cold. J. Hyg. **41:** 225, 1941.

(27) Catchpole, H. R. and I. Gersh. Physiological factors affecting the production of gas bubbles in rabbits decompressed to altitude. J. Cell. and Comp. Physiol. **27:** 15, 1946.

(28) Catchpole, H. R. and I. Gersh. Bubble formation in rabbits decompressed to altitude: effect of preoxygenation, electrical stimulation, and some pharmacological factors. J. Cell. and Comp. Physiol. **27:** 27, 1946.

(29) Catsaras. Cited by Heller, Mager and v. Schrötter, 1886.

(30) Chase, W. H. Anatomical and experimental observations on air embolism. Surg., Gynec. and Obstet. **49:** 569, 1934.

(31) Clark, E. and M. J. Synnott. Influenza-pneumonia cases showing gas in fascial tissues. Am. J. Med. Sci. **157:** 219, 1919.

(32) Coley, B. L. and M. Moore, Jr. Caisson disease with special reference to the bones and joints. Ann. Surg. **111:** 1065, 1940.

(33) Cobb, S. Cerebral circulation: a critical discussion of the symposium. Assn. Res. Nerv. and Ment. Disease **18:** 719, 1938.

(34) Cobb, S. and J. H. Talbot. Studies in cerebral circulation. II. A quantitative study of cerebral capillaries. Trans. Assn. Amer. Phys. **42:** 255, 1927.

(35) Craigie, E. H. The comparative anatomy and embryology of the capillary bed of the central nervous system. Assn. Res. Nerv. and Ment. Disease **18:** 3, 1938.

(36) Curtillet, E. L'embolie gazeuse artérielle. J. de Chirurg. **53:** 461, 1939.

(37) Daly, I. de B., P. L. Eggleton, S. R. Elsden, C. O. Hebb and O. A. Trowell. The significance of safe decompression rates for divers in relation to safe rates of ascent for airmen with special reference to the effect of explosive decompression. Flying Personnel Research Committee, Rept. no. 200, Nov. 1940.

(38) Dean, R. B. The formation of bubbles. J. Appl. Physics **15:** 446, 1944.

(39) Dolgopol, V. B. and M. E. Stern. Interstitial emphysema of the lung with spontaneous pneumothorax and subcutaneous emphysema. Arch. Otolaryngol. **31:** 140, 1940.

(40) Dumke, P. R. and C. F. Schmidt. Quantitative measurements of cerebral blood flow in the macaque monkey. Am. J. Physiol. **138:** 421, 1943.

(41) Dunn, J. S. The effects of multiple embolism of pulmonary arterioles. Quart. J. Med. **13:** 129, 1920.

(42) Duyff, J. W. and Bouman. Zellforsch. **5:** 596, 1927, cited from Krogh.

(43) Eggleton, P., S. R. Elsden, J. Fegler and C. O. Hebb. A study of the effects of rapid decompression in certain animals. J. Physiol **104:** 129, 1945.

(44) End, E. The use of new equipment and helium gas in a world record dive. J. Ind. Hyg. and Toxicol. **20:** 511, 1938.

(45) Engel, G. L., J. Romano, J. P. Webb, E. B. Ferris, Jr., H. W. Ryder and M. A. Blankenhorn. Absence of demonstrable injury to the central nervous system

after repeated experiencing of decompression sickness. Committee on Aviation Medicine, Report no. 263, National Research Council, March 1, 1944.

(46) ERDMAN, S. The acute effects of caisson disease or aeropathy. J. Med. Sci., N.S. **145:** 520, 1913.

(47) ESSEX, H. E., J. F. HERRICK, E. J. BALDES AND F. C. MANN. Influence of exercise on blood pressure, pulse rate, and coronary blood flow of the dog. Am. J. Physiol. **125:** 614, 1939.

(48) FERRIS, E. B., W. E. MOLLE AND H. W. RYDER. Nitrogen exchange in tissue components of man. Committee on Aviation Medicine, Report no. 60, National Research Council, July 15, 1942.

(49) FEGLER, J. Observations on guinea-pigs explosively decompressed to equivalent heights of 41,000 to 45,000 feet. Flying Personnel Research Committee, no. 349, August, 1941.

(50) FEGLER, J., C. HEBB AND W. MISSIURO. Postmortem conditions of the lung characteristic of deaths at high altitudes. Flying Personnel Research Committee, no. 575, February, 1944.

(51) GERSH, I. AND H. R. CATCHPOLE. Appearance and distribution of gas bubbles in rabbits decompressed to altitude. J. Cell. and Comp. Physiol. in press, 1946.

(52) GERSH, I., G. E. HAWKINSON AND E. N. RATHBUN. Tissue and vascular bubbles after decompression from high pressure atmospheres—correlation of specific gravity with morphological changes. J. Cell. and Comp. Physiol. **24:** 35, 1944.

(53) GERSH, I., G. E. HAWKINSON AND E. H. JENNEY. Comparison of vascular and extravascular bubbles following decompression from high pressure atmospheres of oxygen, helium-oxygen, argon-oxygen and air. J. Cell. and Comp. Physiol. **26:** 63, 1945.

(54) GERSH, I. AND M. A. STILL. Blood vessels in fat tissue. Relations to problems of gas exchange. J. Exper. Med. **81:** 219, 1945.

(55) GERSH, I. Pneumothorax and extrapulmonic emphysema in cats exposed to oxygen under pressure. Project X-192, Report no. 5, Naval Medical Research Institute, October 30, 1944.

(56) GERSH, I. Gas bubbles in bone and associated structures, lung and spleen of guinea pigs decompressed rapidly from high pressure atmospheres. J. Cell. and Comp. Physiol. **26:** 101, 1945.

(57) GERSH, I. Correlation of x-ray and gross observations on gas bubbles in guinea pigs decompressed from high pressure atmospheres. J. Cell. and Comp. Physiol., in press, 1946.

(58) GILDEA, E. F. AND S. COBB. The effects of anemia on the cerebral cortex of the cat. Arch. Neurol. and Psychiat. **23:** 876, 1930.

(59) GOGGIO, A. F. AND G. H. HOUCK. Physiologic abnormalities and pathologic changes following exposure to simulated high altitudes. War Med. 7: 152, 1945.

(60) GRAY, J. S., R. L. MASLAND AND S. C. F. MAHADY. The effects of breathing carbon dioxide in oxygen on altitude decompression sickness. AAF School of Aviation Medicine, Proj. no. 409, Report no. 1, July 23, 1945.

(61) GREEN, H. D., R. N. LEWIS, N. D. NICKERSON AND A. L. HELLER. Blood flow, peripheral resistance and vascular tonus, with observations on the relationship between blood flow and cutaneous temperature. Am. J. Physiol. **141:** 518, 1944.

(62) GRIFFIN, D. R., S. ROBINSON, H. S. BELDING, R. C. DARLING AND E. TURRELL. The effects of cold and rate of ascent on aero-embolism. Committee on Aviation Medicine, Report no. 174, National Research Council, June 22, 1943.

(63) GRINDLAY, J. H., J. F. HERRICK AND F. C. MANN. Measurement of the blood flow of the liver. Am. J. Physiol. **132:** 489, 1941.

(64) HAGGART, G. R. AND A. M. WALKER. The physiology of pulmonary embolism as disclosed by quantitative occlusion of the pulmonary artery. Arch. Surg. **6:** 762, 1923.

(65) HALDANE, J. S. AND J. G. PRIESTLEY. Respiration. New Haven, Yale University Press, 1935.

(66) HALDI, J., G. GIDDINGS AND W. WYNN. Dietary control of the water content of the skin of the albino rat. Am. J. Physiol. **135:** 392, 1942.

(67) HAMMAN, L. Spontaneous mediastinal emphysema. Bull. Johns Hopkins Hosp. **64:** 1, 1939.

(68) HARRIS, M., W. E. BERG, D. M. WHITAKER AND V. C. TWITTY. The relation of exercise to bubble formation in animals decompressed to sea level from high barometric pressures. J. Gen. Physiol. **28:** 241, 1945.

(69) HARRIS, M., W. E. BERG, D. M. WHITAKER, V. C. TWITTY AND L. R. Blinks. Carbon dioxide as a facilitating agent in the initiation and growth of bubbles in animals decompressed to simulated altitudes. J. Gen. Physiol. **28:** 225, 1945.

(70) HARVEY, E. N., W. D. MCELROY, A. H. WHITELEY, G. H. WARREN AND D. C. PEASE. Bubble formation in animals; analysis of gas tension and hydrostatic pressure in cats. J. Cell. and Comp. Physiol. **24:** 117, 1944.

(71) HARVEY, E. N. Decompression sickness and bubble formation in blood and tissues. Bull. N. Y. Acad. Med., N.S. **21:** 505, 1945.

(72) HARVEY, E. N., D. K. BARNES, W. D. MCELROY, A. H. WHITELEY, D. C. PEASE AND K. W. COOPER. Bubble formation in animals; physical factors. J. Cell. and Comp. Physiol. **24:** 1, 1944.

(73) HARVEY, E. M., A. H. WHITELEY, W. D. MCELROY, D. C. PEASE AND D. K. BARNES. Bubble formation in animals; Gas nuclei and their distribution in blood and tissues. J. Cell. and Comp. Physiol. **24:** 23, 1944.

(74) HAWKINS, J. A. AND C. W. SHILLING. Nitrogen solubility in blood at increased air pressures. J. Biol. Chem. **113:** 273, 1936.

(75) HAWKINS, J. A. AND C. W. SHILLING. Helium solubility in blood at increased pressures. J. Biol. Chem. **113:** 649, 1936.

(76) HAWKINS, J. A., E. W. SHILLING AND R. A. HANSEN. A suggested change in calculating decompression tables for diving. U. S. Nav. Med. Bull. **33:** 327, 1935.

(77) HEBB, C. O. Observations on gas bubble formation and lung damage in animals rapidly decompressed to 43,000–47,000 feet. Flying Personnel Research Committee, no. 316, June 1941.

(78) HEBB, C. O. Conditions relating to the resistance of animals to high altitudes. Flying Personnel Research Committee, no. 544, August 1943.

(79) HEBB, C. O. A critical effect of temperature in rapid decompression of rats to 44,000 feet. Flying Personnel Research Committee, no. 625, December 1944.

(80) HELLER, R., W. MAGER AND H. V. SCHRÖTTER. Luftdruckerkrankungen. Wien: Alfred Holder, 1900.

(81) HILL, L. AND M. GREENWOOD. The influence of increased barometric pressure in man. No. 3. The possibility of oxygen bubbles being set free in the body. Proc. Roy. Soc., London B **79:** 284, 1907.

(82) HILL, L. AND M. GREENWOOD. Cited by L. Hill. Caisson sickness. New York, Longmans, Green & Co., 1912.

(83) HILL, L. AND J. J. R. MACLEOD. The influence of compressed air on the respiratory exchange. J. Physiol. **29:** 492, 1903.

(84) HOPPE-SEYLER. Cited by Heller, Mager and v. Schrötter, 1857.

(85) HUGGINS, C. AND B. H. BLOCKSON, JR. Changes in outlying bone marrow accompanying a local increase of temperature within physiological limits. J. Exper. Med. **64:** 253, 1936.

(86) HUTCHINS, H. C., A. Y. WERNER, O. E. REYNOLDS AND F. R. PHILBROOK. A study of aerodontalgia occurring during routine oxygen indoctrination in the low pressure chamber with a view to evolving a theory regarding its cause. U. S. M. C. Air Station, Medical Department, Quantico, Va., July 23, 1945.

(87) Ivy, A. C., A. J. Atkinson, H. Adler and W. Burkhardt. Pertaining to the effect of B²B (dextroamphetamine) and of preoxygenation plus B²B on the incidence of "bends" and "incapacitating bends and chokes" at 40,000 feet for one hour. Committee on Aviation Medicine. Report no. 113, National Research Council, December 23, 1942.

(88) Jacobs, M. H. and D. R. Stewart. Observations on the blood of albino rats following rapid decompression. Committee on Aviation Medicine, Report no. 76, National Research Council, October 1942.

(89) Jones, R. R., J. W. Crossin, E. E. Griffith, R. R. Sayers, H. H. Schrenk and F. Levy. Administration of pure oxygen to compressed air workers during decompression: prevention of the occurrence of severe compressed illness. J. Ind. Hyg. and Toxicol. **22:** 427, 1940.

(90) Jones, H. B., W. E. Myers and W. E. Berg. Gas exchange, circulation and diffusion. Committee on Aviation Medicine, Report no. 429, National Research Council, April 10, 1945.

(91) Jones, H. B., C. Tobias, W. F. Loomis, J. B. Mahoney, W. N. Sears, J. C. Larkin, J. G. Hamilton and J. H. Lawrence. An objective method for the study of the physiology of aeroemphysema and for the selection of high altitude aircrew using the radioactive isotopes of inert gases. Committee on Aviation Medicine, Report no. 81, National Research Council.

(92) Kabat, H. and C. Dennis. Decerebration in the dog by complete temporary anemia of the brain. Proc. Soc. Exper. Biol. and Med. **38:** 864, 1938.

(93) Kabat, H., C. Dennis and A. B. Baker. Recovery of function following arrest of the brain circulation. Am. J. Physiol. **132:** 737, 1941.

(94) Kabat, H. and M. Schadewald. The relative susceptibility of the synaptic terminals and of the parenchyma to arrest of the circulation of the brain. Am. J. Path. **17:** 833, 1941.

(95) Kahlstrom, S. C., C. C. Burton and D. B. Phemister. Aseptic necrosis of bone I. Infarction of bones in caisson disease resulting in encapsulated and calcified areas in diaphyses and in arthritis deformans. Surg., Gynec. and Obstet. **68:** 129, 1939.

(96) Kelman, S. Experimental emphysema. Arch. Int. Med. **24:** 332, 1919.

(97) Kenrick, F. B., C. S. Gilbert and K. L. Wismer. The super-heating of liquids. J. Phys. Chem. **28:** 1297, 1924.

(98) Kenrick, F. B., K. L. Wismer and K. S. Wyatt. Supersaturation of gases in liquids. J. Phys. Chem. **28:** 1308, 1924.

(99) Knisely, M. H., S. Gray, H. M. Peck, R. L. Nichols, L. Warner and J. A. Orcutt. The effect of elevation of a limb on the development and severity of bends pain. Committee on Aviation Medicine, Report no. 196, National Research Council, October 1, 1943.

(100) Knisely, M. H., S. Gray, H. M. Peck, R. L. Nichols, L. Warner, J. A. Orcutt and N. Anderson. Preliminary tests of the effect of intravenous aminophyllin in preventing or alleviating bends and chokes. Committee on Aviation Medicine, Report no. 195, National Research Council, October 1, 1943.

(101) Krogh, A. The anatomy and physiology of capillaries. New Haven, Yale University Press, 1929.

(102) Kuper, J. B. H. Some remarks on the formation and resolution of bubbles (with special reference to aeroembolism). Report no. 4, Studies in Aviation Medicine, Research Section of Division of Industrial Hygiene, National Institute of Health. January 3, 1942.

(103) Ladewig, P. Anoxaemic changes in the liver, with regard to the "high altitude death" of airmen. Nature **151:** 558, 1943.

(104) Lazarow, A., P. R. Patek, E. Bartosh and G. H. Scott. Observations on the capillary circulation in skeletal muscle of frogs at simulated high altitudes. Committee on Aviation Medicine, Report no. 162, National Research Council, June 15, 1943.

(105) LUND, D. W. AND J. H. LAWRENCE. An hypothesis as to a cause of "bends" pain with observations on massage at high altitude. Committee on Aviation Medicine, Report no. 404, National Research Council, January 10, 1945.

(106) LUND, D. W., J. H. LAWRENCE AND L. B. LAWRENCE. Latent neurological manifestations following decompression. Occupational Medicine **1**: 75, 1946.

(107) MACKLIN, C. C. Pneumothorax with massive collapse from experimental local overinflation of the lung substance. Can. M. A. J. **36**: 414, 1937.

(108) MACKLIN, C. C. Transport of air along sheaths of pulmonic blood vessels from alveoli to mediastinum. Arch. Int. Med. **64**: 913, 1939.

(109) MCELROY, W. D., A. H. WHITELEY, K. W. COOPER, D. C. PEASE, G. H. WARREN AND E. N. HARVEY. Bubble formation in animals; physiological factors: the rôle of circulation and respiration. J. Cell. and Comp. Physiol. **24**: 273, 1944.

(110) MCELROY, W. D., A. H. WHITELEY, G. H. WARREN AND E. N. HARVEY. Bubble formation in animals; relative importance of carbon dioxide concentration and mechanical tension during muscle contraction. J. Cell. and Comp. Physiol. **24**: 133, 1944.

(111) MCGUIRE, J. AND W. B. BEAN. Spontaneous interstitial emphysema of the lungs. Am. J. Med. Sci. **197**: 502, 1939.

(112) Mediastinal emphysema (pneumomediastinum) following explosive decompression of humans; report of two cases. Jan. 1, 1945, Memorandum Report of Aero Medical Laboratory, Wright Field, Dayton, Ohio, no. TSEAL-3-695-29-I.

(113) MERINGI, G., G. H. v. SCHOTTZ AND J. GYURIK. Über die Wirkung von Ammonium Chloride auf die Hohenfestigkeit des Menschen. Luftfahrtmed. **5**: 102, 1941.

(114) MITCHELL, D. F. Aerodontalgia. Bull. U. S. Army Med. Dept., no. 73, 62, 1944.

(115) MORALES, M. F., E. N. RATHBUN, R. E. SMITH AND N. PACE. Studies on body composition: theoretical considerations regarding major body tissue components, with suggestions for application to man. J. Biol. Chem. **158**: 677, 1945.

(116) MORALES, M. F. AND R. E. SMITH. On the possible determination of gross human body composition by the use of radioactive inert gases. Research Project X-43, Report no. 4, Naval Medical Research Institute, August 1, 1945.

(117) MORALES, M. F. AND R. E. SMITH. The physiological factors which govern inert gas exchange. Bull. Math. Biophys. **7**: 99, 1945.

(118) MORALES, M. F. AND R. E. SMITH. On the theory of blood-tissue exchanges: III. Circulation and inert gas exchanges at the lung with special reference to saturation. Bull. Math. Biophys. **6**: 141, 1944.

(119) MORALES, M. F. AND R. E. SMITH. A note on the physiological arrangement of tissues. Bull. Math. Biophys. **7**: 47, 1945.

(120) MOREY, J. B. AND M. C. SOSMAN. Spontaneous mediastinal emphysema, with report of case associated with spontaneous pneumothorax. Radiology **32**: 19, 1939.

(121) OSIPOV, M. O. AND V. F. LASHKOV. A contribution to the pain problem at high altitude. Transl. by S. Kohn, R. C. A. M., July 15, 1940.

(122) OUDARD. Accidents de décompression: Relation d'autopsie. Arch. de Méd. et Pharm. Nav. **95**: 63, 1911.

(123) PACE, N. Equations for the estimation of total body fat and total body water from the solubility of inert gases in the body. Research Project X-191, Report no. 4, Naval Medical Research Institute, September 25, 1945.

(124) PAPPENHEIMER, J. R. AND J. P. MAES. The effects of vasoconstriction on the apparent viscosity of blood flowing through the hindlimb of the dog. Federation Proc. **1**: 65, 1942.

(125) PATEK, P. R., A. LAZAROW, E. BARTOSH AND G. H. SCOTT. Observation on living periosteum and peritoneum at simulated high altitudes. Committee on Aviation Medicine. Report no. 244, National Research Council, January 17, 1944.

(126) PEASE, D. C., L. R. BLINKS AND E. A. REED. Bubble formation in decompressed

animals. VII. Physical factors in bubble formation. Committee on Aviation Medicine, Final Report OEMcmr-193, National Research Council, October 15, 1944.

(127) PHEMISTER, D. B. Changes in bones and joints resulting from interruption of circulation. II. Non-traumatic lesions in adults with bone infarction; arthritis deformans. Arch. Surg. **41:** 1455, 1940.

(128) PICCARD, J. Aeroemphysema and the birth of gas bubbles. Proc. Staff. Meetings, Mayo Clinic **16:** 700, 1941.

(129) PIGOTT, J. P. Dental pain at high altitudes; origin and treatment. Interim Report OEMcmr-38, National Research Council, October 23, 1944.

(130) POLAK, I. B. AND B. H. ADAMS. Traumatic air embolism in submarine escape training. U. S. Nav. Med. Bull. **30:** 165, 1932.

(131) POLAK, I. B. AND C. L. TIBALS. A fatal case of caisson disease following a dive of short duration to a depth of thirty feet. U. S. Nav. Med. Bull. **28:** 862, 1930.

(132) PUDENZ, R. H. Personal communication.

(133) R.A.F. Institute of Aviation Medicine, Physiological Research Unit, Farnsborough, England. Observations on Decompression Sickness in Man. Flying Personnel Research Committee Rept. no. 267. March, 1941.

(134) RATHBUN, E. N. AND N. PACE. Studies on body composition. I. The determination of total body fat by means of the body specific gravity. Research Project X-191, Report no. 1, Naval Medical Research Institute, August 7, 1944.

(135) RATNOFF, O. D. The absence of roentgenographically demonstrable bony changes at the hip joint in subjects exposed to simulated high altitudes. School of Aviation Medicine, Randolph Field, Texas, Report no. 201, November 12, 1943.

(136) Recommendation for the handling of reactions following altitude chamber flights. A. A. F. School of Aviation Medicine, Randolph Field, Texas, Project no. 217, Report no. 1, December 30, 1943.

(137) REED, E. A. AND L. R. BLINKS. Bubble formation in decompressed animals. V. The relation of temperature and exercise to bubble formation in rats, and in tourniqueted legs of rabbits and goats. Final Report no. 377, National Research Council, October 15, 1944.

(138) REED, E. AND L. R. BLINKS. Bubble formation in decompressed animals. VI. Vasoconstriction and the relation of the vascular bed to bubble formation in frogs. Committee on Aviation Medicine, Report no. 379, National Research Council, October 15, 1944.

(139) RENDICH, R. A. AND L. A. HARRINGTON. Roentgen findings in caisson disease of bone, with case reports. Radiology **35:** 439, 1940.

(140) RICHARDSON, H. F., B. C. COLES AND G. E. HALL. Experimental gas embolism. I. Intravenous air embolism. Canad. M. A. J. **36:** 584, 1937.

(141) RYDER, H. W., G. L. ENGEL, J. ROMANO, J. P. WEBB, M. A. BLANKENHORN, E. B. FERRIS AND W. E. BROWN. An assay of dextro-amphetamine for its protective value in decompression sickness. Committee on Aviation Medicine, Report no. 112, National Research Council, January 28, 1943.

(142) SCHMIDT, C. F., S. S. KETY AND H. H. PENNES. The gaseous metabolism of the brain of the monkey. Report no. 389, OEMcmr-28, National Research Council, September 1, 1944.

(143) SCHULTZ. Cited by Heller, Mager and v. Schrötter, 1878.

(144) SENDROY, J., Jr., R. T. DILLON AND D. D. VAN SLYKE. Studies of gas and electrolyte equilibrium in the blood. XIX. The solubility and physical state of uncombined oxygen in the blood. J. Biol. Chem. **105:** 597, 1934.

(145) SHARPLESS. Cited by Heller, Mager and v. Schrötter.

(146) SHAW, L. A., A. R. BEHNKE, A. C. MESSER, R. M. THOMSON AND E. P. MOTLEY. The equilibrium time of the gaseous nitrogen in the dog's body following changes of nitrogen tension in the lungs. Am. J. Physiol. **112:** 545, 1935.

(147) SJÖSTRAND, T. On the principles for the distribution of the blood in the peripheral vascular system. Skand. Arch. f. Physiol. **71:** suppl., 1935.

(148) SMITH, H. The physiology of the kidney. New York: Oxford University Press, 1937.

(149) SMITH, J. J. Effects of explosive decompression on animals. War Department, Air Corps Materiel Division, Report no. EXP-M-54-653-34D, May 20, 1942.

(150) SMITH, R. E. AND M. F. MORALES. On the theory of blood tissue exchange: I. Fundamental equations. Bull. Math. Biophys. **6:** 125, 1944.

(151) SMITH, R. E. AND M. F. MORALES. On the theory of blood tissue exchanges: II. Applications. Bull. Math. Biophys. **6:** 133, 1944.

(152) SNYDER, C. D. Recent advances in knowledge of the liver. Physiol. Rev. **22:** 54, 1942.

(153) STOEL, G. Zellforsch. **3:** 91, 1925, cited from Krogh.

(154) SWINDLE, P. F. ET AL. The possible relationship between intravascular agglutination of erythrocytes and decompression sickness. Committee on Aviation Medicine, Report no. 178, National Research Council, August 13, 1943.

(155) THOMAS, S. AND O. L. WILLIAMS. High altitude joint pains: their radiographic aspects. Committee on Aviation Medicine, Report no. 395, National Research Council, December 11, 1944.

(156) THUDICHUM, T. L. W. Die Chemische Konstitution des Gehirns der Menschen und der Thiere. Tübingen: F. Putzcker, 1901.

(157) TOBIAS, W. F., W. F. LOOMIS, F. C. HENRY, W. R. LYONS, H. B. JONES, W. N. SEARS, S. F. COOK, J. B. MOHNEY, J. G. HAMILTON AND J. H. LAWRENCE. Circulation and decompression sickness. Committee on Aviation Medicine, Report no. 144, National Research Council, June 7, 1943.

(158) TROWELL, O. A. Liver vacuoles and anoxia. Nature **151:** 730, 1943.

(159) TROWELL, O. A. Histological changes in the lungs of rabbits decompressed to 40,000–47,000 feet. Flying Personnel Research Committee, no. 317, June 1941.

(160) TROWELL, O. A. A histological examination of the spinal cord of animals rapidly decompressed to 40,000–45,000 feet. Flying Personnel Research Committee, no. 345, August, 1941.

(161) TURPIN, F. H., W. F. LOOMIS, J. H. LAWRENCE, H. B. JONES AND C. A. TOBIAS. Solubilities of gases in water and oils. Report no. 455, National Research Council, July, 1945.

(162) TUREEN, L. L. AND J. B. DEVINE. The pathology of air embolism. J. Missouri State Med. Assn., 33, 141, 1936.

(163) TWYNAM, G. E. A case of caisson disease. Brit. Med. J. **1:** 190, 1888.

(164) UNDERWOOD, N. AND J. T. DIAZ. A study of the gaseous exchange between the circulatory system and the lungs. Am. J. Physiol. **133:** 88, 1941.

(165) VAN ALLEN, C. M., G. L. NOCOLL AND W. M. TUTTLE. Lung changes after occlusion of pulmonary artery branches by embolus and by ligature. Yale J. Biol. and Med. **2:** 363, 1929–30.

(166) VAN RENSSELAER, H. The pathology of the caisson disease. Med. Rec. **40:** 141, 178, 1891.

(167) VAN SLYKE, D. D., R. T. DILLON AND R. MARGARIA. Studies of gas and electrolyte equilibria in blood. XVIII. Solubility and physical state of atmospheric nitrogen in blood cells and plasma. J. Biol. Chem. **105:** 571, 1934.

(168) VERNON, H. M. The solubility of air in fats and its relation to caisson disease. Proc. Roy. Soc. London B **79:** 366, 1907.

(169) VIGUIER AND G. JEAN. Gaseous embolism of the gluteal artery (produced by decompression). Bull. Acad. de Med. **80:** 377, 1918.

(170) VILLARET, M., R. CACHERA AND R. FAUVERT. L'embolie gazeuse cérébrale; ses effets circulatoires locaux. C. R. Soc. de Biol. **125:** 108, 1937.

(171) v. Leyden. Cited by Heller, Mager and v. Schrötter, 1877.
(172) Wagner, C. E. Observations of gas bubbles in pial vessels of cats following rapid decompression from high pressure atmospheres. J. Neurophysiol. **8:** 29, 1945.
(173) Warren, C. O., Jr. The oxygen consumption of rabbit bone marrow in relation to its morphology. Am. J. Physiol. **110:** 61, 1934.
(174) Webb, J. P., E. G. Ferris, Jr., L. Engel, J. Romano, H. W. Ryder, C. D. Stevens and M. A. Blankenhorn. Radiographic studies of the knee during bends. Committee on Aviation Medicine, Report no. 305, National Research Council, May 8, 1944.
(175) Wearn, J. T. Morphological and functional alterations of the coronary circulation. The Harvey Lectures, 243, 1939–40.
(176) Weinberger, L. M., M. H. Gibbon and J. H. Gibbon, Jr. Temporary arrest of the circulation to the central nervous system. Arch. Neurol. and Psychiat. **43:** 615, 1940.
(177) Whitaker, D. M., L. R. Blinks, W. E. Berg, V. C. Twitty and M. Harris. Muscular activity and bubble formation in animals decompressed to simulated altitudes. J. Gen. Physiol. **28:** 213, 1945.
(178) Whitaker, S. R. F. and F. R. Winton. The apparent viscosity of the blood flowing in the isolated hind limb of the dog, and its variation with corpuscular concentration. J. Physiol. **78:** 339, 1933.
(179) Whiteley, A. H., W. D. McElroy, G. H. Warren and E. N. Harvey. Bubble formation in animals; denitrogenation. J. Cell. and Comp. Physiol. **24:** 257, 1944.
(180) Windle, W. F., R. F. Becker and A. Weil. Alterations in brain structure after asphyxiation at birth. An experimental study in the guinea pig. J. Neuropath. and Exper. Neurol. **3:** 224, 1944.
(181) Wolff, H. G. The cerebral blood vessels—anatomical principles. Assn. Res. Nerv. and Ment. Dis. **18:** 29, 1938.
(182) Yaskin, J. C. and M. W. Thorner. The effects upon the cerebral cortex of altitude chamber anoxia. Trans. Am. Neurol. Assn. **69:** 88, 1943.
(183) Zetterström, A. Deep sea diving with synthetic gas mixtures. Report from the Swedish Admiralty, 1944.
(184) Zografidi, S. Contribution à l'étude des accidents de decompression chez les plongeurs à scaphandre. Rev. de Med. **27:** 159, 1907.
(185) Zuntz, N. Zur Pathogenese und Therapie der durch rasche Luftdruckänderung erzeugten Krankheiten. Fortschritte der Med. XV Jahr., 632, 1897.

THE GENESIS OF THE ELECTROCARDIOGRAM

LOUIS N. KATZ

The Cardiovascular Department, Research Institute, Michael Reese Hospital, Chicago, Ill.[1]

A review of the significance of the electrocardiogram may present an extensive noncommittal report of a voluminous literature or a summary of the author's viewpoint based on a fair evaluation of the pertinent studies of others and on his own experiences and deductions. The latter approach was chosen. Several excellent reviews have appeared (10, 13, 27, 61, 153, 155, 162, 163, 294, 299, 309, 314, 356, 367) since the last PHYSIOLOGICAL REVIEW article written by the author in 1928 (161). The present review will concern itself only with the theoretical aspects of the electrocardiogram. The empirical interpretations of the electrocardiogram which have been found so useful in clinical practice will not be discussed since they have been dealt with in extenso elsewhere (164).

Any careful survey of the articles appearing on the subject must impress the unbiased reader with the fact that the manifold theories on the genesis of the electrocardiogram are in apparent contradiction with one another. He may perforce in desperation apply electrocardiography without regard to theory, leaving the answer to the latter to the future. It is very fortunate that the clinical usefulness of the electrocardiograph has suffered so little from lack of agreement on its theoretical aspects. While some workers continue to proclaim the need for an understanding of the theory in order to make electrocardiography useful for clinical practice, the electrocardiograph has actually been very intensively and profitably used without any but the most elementary theoretical background. It may be stated in all fairness that the attempt to instill a theoretical grounding into the ordinary clinician who wishes to use this tool has served too often to confuse rather than to assist him. This is so because the background of physics, mathematics and physiology which the ordinary electrocardiographer possesses is too superficial for a critical evaluation of the facts and fancies presented. Too many presentations of the subject make untenable assumptions of the constancy of certain variables in order to arrive at a "simple" mathematical synthesis of the subject. Some recognize the assumptions made at the start but too often lose sight of them in later developments, especially when the subject is taken over by enthusiastic and worshipful disciples. Having once established a point of view, investigators often continue to expand it until the superstructure of the theory becomes too heavy for the narrow pivot of fact upon which it rests. This attempt to make the interpretation of data yield more precise quantitative information than is justified by the crude initial assumptions, is the basic fault in many instances. It is hoped that this will become apparent as this review is developed.

What has just been stated does not apply to the electrocardiographic interpretation of cardiac arrhythmias. Here knowledge of the normal and abnormal

[1] The department is supported in part by the Michael Reese Research Foundation.

physiology of impulse origin and impulse spread, of the refractory period of the heart and of its syncytial character is of vital importance. The more the electrocardiographer understands these fundamental properties, the more rational become his interpretations. However, this review will not deal with the arrhythmias except in so far as such knowledge helps account for the creation of electric currents by the heart.

Factors Involved in the Genesis of the Electrocardiogram. The genesis of the electrocardiogram is but a special and perhaps more complex case of cellular electrophysiology. Its features are therefore basic in general physiology. However, any analysis of the electrocardiogram must concern itself with the fact that the electrodes used for recording currents are applied to the body surface at a distance from the heart. Even when one of the electrodes is placed over the precordium it is still a distant electrode. Consequently, four principal factors are involved in this analysis:

a. The genesis and typical time course of the electric states of the heart during activity and injury.

b. The laws governing the spread of current within a volume conductor.

c. The influence exerted by the characteristics of the body as a conductor of electricity.

d. The modifications introduced by variations of the anatomy and physiology of the heart.

The Genesis and Typical Time Course of the Electric States of the Heart during Activity and Injury. A. The electric state of resting heart muscle. It is now well established that (a) the composition of living cells differs from that of the ambient extra-cellular fluid (72), and (b) that the surface membrane of cells is semipermeable. It follows, therefore, that the resting cell surface is electrically polarized; this polarity is such that the charge on the inside is negative and that on the outside is positive. It is not unlikely that other electric charges exist at interfaces within cells as complex as that of the heart cell syncytium, but there is no reason to believe that these are involved in the genesis of the electric current recorded in the electrocardiogram. The electric characteristics of the cell surface may not be as simple as suggested above. For example, Cole (59, 59A) has shown that during flow of current across a surface membrane it possesses electric properties of impedance, capacitance and inductance. The classical hypothesis of the polarized membrane, however, is adequate for an understanding of the genesis of the electrocardiogram. The magnitude of the potential across the resting cell membrane was found to be of the order of 100 millivolts in certain cells in which it can be measured (62, 63). However, at rest since, all parts of the cell membrane are maintained uniformly polarized, no potential differences will exist within the cell nor in the medium surrounding the cell, and no electric currents will flow.

B. The alterations of the electric state during activity. It is now generally agreed that during activity of a living cell, the permeability of its surface is altered, the impedance across the surface changes, the ions which are responsible for the polarized state of the resting cell migrate and that the cell becomes

depolarized. Recently Cole (63) observed that following depolarization a temporary reversal of polarization occurs. If confirmed, this would show that the process of local activation is not as simple as has been hitherto conceived. This can be neglected for our purposes since the magnitude of potential differences and current involved is much smaller than that due to depolarization of the "resting" charges.

The process of depolarization in any one region is not instantaneous but of measurable duration. This time plus that occupied in the propagation of the depolarization process throughout the cell surface determine the duration of the process of depolarization. Since during this period the electric state of the cell membrane is not uniform, differences in potential are established within the cell and also in any conducting medium surrounding the cell, and electric currents will flow while these potential differences exist.

When the depolarization is completed the cell may remain in the depolarized state for a variable time. During this time there is no reason to believe that any potential differences exist and hence no electric currents will flow. Thus the cell is electrically quiescent even though it may, as in the case of a contractile organ like the heart, be undergoing changes in tension and in length.

The process of repolarization, which follows soon after depolarization, is definitely slower than the process of depolarization. Further, the pattern of depolarization need not have the same topographical distribution as that of the depolarization preceding it. The evidence is clear that in the case of the heart the duration of electrical systole, that is, the period from the beginning of depolarization until repolarization is completed, is unequal in various sites of the ventricles (cf. 164). Since the electric state of the cell is not uniform during repolarization, potential differences are again established and electric current flows once more within the cell and in any conducting medium surrounding the cell. However, the potential differences are now not as great as during depolarization since the process of repolarization is slower. If the topography of repolarization in the cell were identical with that of depolarization and if the duration of electrical systole were precisely the same in all parts of the cell (unlikely occurrences in the normal ventricles), then the potential differences during repolarization would be opposite in sign to those in the stage of depolarization and the area subtended by it would equal that subtended during depolarization.

The theory outlined above is widely accepted by students of electrocardiography. It is also agreed that these electric phenomena are not manifestations of the mechanical responses of heart muscle, since the time pattern of their occurrence is not identical with that of the mechanical phases (175, 339, 341). The details of the energetics and chemical factors involved in the process leading to electric manifestations (223, 224) are not yet fully understood, but are not the concern of a review which deals only with the electric phenomena themselves. For the genesis of the electrocardiogram it suffices to recognize the following four states of the cell, viz.: *a*, the polarized state of rest; *b*, the state of depolarization—during activation; *c*, the depolarized state of the active cell,

and *d*, the stage of repolarization—during restitution (or recovery). Further, it is important to appreciate that only during the process of depolarization and repolarization do potential differences appear and electric currents flow. The electrogram thus records a double response during the heart cycle when the electrodes are placed on two uninjured parts of the heart's surface, an initial large and brief deflection and a longer and smaller final deflection, with an interval between them. This is the genesis of the P and T_A waves of the auricles and of the QRS complex, S-T segment and T wave of the ventricles in the electrocardiogram.

C. The alterations of the electric state during injury. Injury involving a part of the cell may do a number of things (cf. 318):

1. Injury may completely depolarize a part of the surface of the affected cell, and hence this region does not share in the activity of the rest of the cell. There will be potential differences during the resting polarized state of the uninjured part of the cell, because the cell membrane will not be uniformly polarized; an injury current of rest will therefore be present. During depolarization of the uninjured part this potential difference will disappear since the cell becomes completely depolarized, only to reappear again during repolarization of the uninjured part. Thus there will be an injury current of rest, but no injury current of activity. An injury of this type will result in a monophasic electrogram during the heart cycle if electrodes leading from an uninjured and an injured part of the heart's surface are connected to a recording galvanometer. The S-T deviations in the electrocardiogram caused by injury can be accounted for on this basis. The application of potassium to the surface of the heart appears to operate in this way (23).

2. Injury may cause a partial, rather than a complete, depolarization of the region of the cell affected, but still leave it responsive. During the resting stage, potential differences between injured and uninjured regions will exist but will be of smaller magnitude than in the case of complete depolarization of the injured part. During activation the injured part will also respond and so the potential differences in the cell will disappear. There will thus be a monophasic curve of smaller magnitude than in the preceding situation. In this case also there will be only an injury current of rest and no injury current of activity. This type of injury is thus a variation of the preceding one.

3. Injury while causing only a partial depolarization of the part of the cell affected may also make this area completely or partially unresponsive. During the resting stage the situation would be similar to that of the preceding cases in producing an injury current of rest, but the injured part of the cell would retain its partially polarized state during activity. Thus, when the rest of the cell is depolarized, potential differences of opposite directions to those previously existing would develop. There would thus be not only an injury current of rest but also one of activity. Again a monophasic curve would be recorded when an injured and an uninjured part of the cell are connected to a galvanometer, and S-T deviations would result in the electrocardiogram. But the monophasic curve could cross the null point, once during depolarization and again during

repolarization. This appears to be the common effect of injury produced with the suction (89, 93) or the pressure electrode (318).

4. Injury may result in unresponsiveness of the injured region without affecting its resting polarization, or injury may lead to a region of block preventing the impulse from reaching some part of the cell. Under these circumstances no injury current would flow during the resting state of the cell. But during the activity stage, the injured, or isolated, part of the cell would retain its surface polarization while the rest of the cell was depolarized. There would thus be an injury current of activity but no injury current of rest. Again a monophasic curve would be recorded when an injured and uninjured (or blocked off) part of the cell were connected to a galvanometer, but it would not cross the null point. It would cause S-T deviations in the electrocardiogram. Cold appears to operate in this manner (23).

5. Finally injury may cause a delay and protraction of the process of repolarization in a responsive and fully polarized or partially depolarized part of the cell. This would lead to an injury current of repolarization which would modify the appearance of the final deflection of the electrogram obtained by connecting the injured and uninjured part of the cell (318). This would account for the T wave of injury in the electrocardiogram. If the injured region were completely polarized at rest, there would be no injury current of rest or activity, but only an injury current of repolarization. If the injured part of the cell were partially depolarized there would be not only an injury current of repolarization but also an injury current of activity. It would account for the frequent combination in the electrocardiogram of S-T deviations with oppositely directed T waves. A variant of this would be the case in which the injured area was partially depolarized, partially unresponsive and also had a protracted period of repolarization. Here there would be an injury current at rest, an injury current during activity and an injury current of repolarization. Injury currents of repolarization may occur in areas of the cell different from those giving rise to the other types of injury currents; usually the two areas are adjacent to each other (318). The amount of delay and protraction in the repolarization of the injured area may vary, accounting for different degrees of injury currents of repolarization and various magnitudes and durations of injury T waves.

The exposition above will account for the genesis of the S-T and T wave deviations which arise as a result of injury. It remains to point out that when a part of the injured cell is partially or wholly unresponsive this will alter the appearance of the initial deflection of the electrogram obtained in connecting an injured with an uninjured area and also of the QRS deflection of the electrocardiogram since the orientation of the potential differences during the process of depolarization of the cell are altered. (Some of the T wave alterations in injury may be on a similar basis; namely, reorientation of the potential differences during repolarization because of absence of response of the injured area.)

The living cell ordinarily does not permit the state of injury to persist but tends to restore the normal state. Sometimes, however, the processes giving rise to injury balance the restitution processes, and the injury state is maintained

for long periods of time. The newly formed part of the polarized surface may enclose the injured area which then is restored to the cell, or it may develop at the junction between the injured and uninjured parts of the cell, the former thus becoming dead tissue. This development of a newly formed surface of polarization causes the injury pattern to change with the passage of time. It also accounts for the tendency for the effects of injury to change from injury currents during rest and/or activity, causing S-T deviations, to injury currents during repolarization, causing T changes. On the other hand, if a mild injury became more severe, the reverse would take place. As soon as recovery from injury is complete, the injury S-T deviations and T wave alterations disappear. Thus it may be stated categorically that S-T deviations and T wave alterations of injury indicate the persistence of injury currents, whereas QRS alterations due to injury need not disappear since they often remain when the injured area has been replaced by necrotic or scar tissue.

Considerable controversy has arisen as to whether it is the injured or the uninjured part of the cell which is responsible for the injury currents (56, 61, 80, 81, 89, 94, 102, 103, 126, 156, 168, 215, 225, 307, 308, 309, 310, 318, 367, 369). The discussion given above shows that both areas are involved. If no injured area is present, no injury current will develop; and likewise if *no* part of the cell remains uninjured, no injury current will occur. Injury currents signify that part of the cell is injured, but not all of it. The source of the current is obviously ultimately referable to that part of the cell which is polarized. Depending on circumstances this may be the uninjured or the injured part, or both, and this can be worked out for the various effects of the injury, as outlined above. Actually, experience shows that the onset, duration and end of the monophasic curve depends on the electrode placed over the uninjured area rather than on that placed over the injured area (156, 309; also cf. 80). The modification introduced when indirect leads, or leads involving one electrode on the surface of the heart and the other at a distance are employed, will be discussed below.

The attempt to read into the monophasic curve a series of phenomena as has recently been done (289, 290, 291, 292) has rightly been criticized as a misinterpretation of artefacts in the record (11A, 80). Even with the best technique the curves obtained may not be pure injury currents, but may contain some distortions, since the electrode over the injured area may pick up currents from uninjured areas in the neighborhood of the injury.

D. The syncytial character of the heart. The heart is composed of a complex of spiral muscles arranged in sheaths and in bundles (262, 279). The muscle bundles of the auricles are distinct from those of the ventricles, and separated from them by a fibrous tissue sheet at the auriculo-ventricular junction to which the heart valves are attached. In the mammal and bird there is ordinarily only one muscular bridge between the two, consisting of neomorphic specialized muscular tissue. This so-called auriculo-ventricular bridge is made up of the auriculo-ventricular node and the common auriculo-ventricular bundle. While some recent workers have questioned the specialized character of this bridge in the dog, monkey and man, because the histological differentiation from ordinary

heart muscle is not as striking as in other species (105, 106, 107, 108), the overwhelming mass of evidence shows its anatomical existence (37, 38, 58, 60, 64, 65, 66, 67, 68, 178, 198, 201, 208, 209, 210, 235, 270, 272, 284, 293, 294, 327, 332, 333, 334, 338, 378, 379) and, more important, shows it to be the sole bridge which permits the transfer of the impulse from the auricles to the ventricles. The recent attempt to revive the hypothesis of the neurogenic transfer of the impulse from the auricles to the ventricles (105, 106, 107, 108) has not presented new evidence. The controversy raging a quarter of a century ago was finally settled in favor of the myogenic hypothesis of impulse transmission, and the evidence today still favors it (198, 294, 327). Of course, it is true that in mammals there may occasionally exist other muscular connections between the auricles and ventricles, like the bundle in the lateral wall of the right heart found by Kent in some herbivora. In fact, a type of electrocardiogram with an abnormally short P-R interval and an abnormally long QRS duration (the Wolff-Parkinson-White syndrome) has recently been explained on the basis of such a congenital by-pass. This by-pass, in various locations, has actually been demonstrated at necropsy in such cases (240, 377). Further, it must be admitted that the auriculo-ventricular connective tissue partition is indented by ventricular and auricular muscle so that it has corrugated surfaces. To elucidate the rôle played in transmission by muscle bundles penetrating the auriculo-ventricular fibrous partition, a systematic survey of human hearts with serial wax model preparations is needed. It appears unlikely that such a survey will alter the classical, widely accepted views of the nature and function of the auriculo-ventricular muscular bridge.

The auricular and ventricular musculature, therefore, each form a single unit, linked together by a narrow muscular bridge. Each may be considered as a syncytium. While it may be argued on histological grounds that some of the cross-striations divide the muscle into distinct cellular compartments, from the point of view of the genesis of the electrocardiogram the muscle fibers of the auricles and those of the ventricles may be considered as each constituting a single though extremely complex syncytial cell. On this hypothesis there is fairly complete agreement.

This concept of the syncytial nature of heart muscle is important in explaining the continuity of the impulse transmission over the various chambers of the heart. It readily explains why an injured region within the heart muscle which does not border on the endocardium or epicardium creates no external electric field. It explains why an impulse spreading radially in all directions from a point within the heart muscle does not create an external electric field until it reaches the endocardial, epicardial or the auriculo-ventricular fibrous partition surfaces. If the myocardium were composed of discrete electrically polarized cells rather than a single syncytial cell each for the ventricles and auricles, then injury entirely within the myocardium, unless it were such as to affect the entire surface of the cells involved, should on some occasion cause an external electric field. On the basis of discretely discharging multiple cells, it would be difficult to account for the deflections occurring during the cycle of cardiac activity. The potential differences occurring during depolarization and repolarization exist only as long

as the cell throughout its surface is not in an identical electric state. There would be no reason to find a set of deflections for auricular activity distinct from those of ventricular activity since it is difficult to see how the spread of depolarization and repolarization over large numbers of discrete cells could summate in such a way as to give the large deflections of the electrical record obtained from the heart. Such problems in explaining the electrocardiogram are avoided by accepting the view that the heart is made up of two cells, one composing the auricles, the other the ventricles.

E. Other sources of electric currents in the heart. From time to time other sources of electricity have been invoked to account for the various deflections of the electrocardiogram (196, 202, 217; cf. 164). Frictional electricity, for example, has been suggested to explain the T wave, although the evidence has never appeared convincing. While movements of the heart and changes in the electrical impedance (253, 288) during the heart cycle may modify the distribution of the electric currents, they cannot be considered as a cause for the electric forces which are created during the heart cycle. The P and T_A waves of the auricles, and the QRS deflection, the S-T segment and the T wave of the ventricles are adequately accounted for on the basis of the polarization hypothesis developed above. One dilemma in the account of the deflections of the electrocardiogram is the causation of the U wave. While a number of suggestions have been made to account for this deflection (138, 226, 227, 243, 312), its exact cause remains to be determined. It is not unlikely that it is similar in character to the after-discharges found in nerve fibers, which occur also in the heart (45, 311).

F. Topography of impulse origin and spread. The various deflections of the electrocardiogram depict the time course of the processes of depolarization and repolarization in the auricles and ventricles. The meaning of this time pattern will become clearer once the spatial pattern of impulse origin and spread as determined by their topography in the heart is clearly understood. In this review only a bare outline of this topography of the heart will be attempted, based on the classical view which remains ascendant despite numerous attacks over the past four decades. The mechanisms of impulse initiation and those of impulse propagation can not be considered here, nor can any detailed account be given of the anatomy of the muscular elements involved.

The impulse originates in the sinus node located in the sulcus terminalis of the right auricle, or more precisely in the head of this node. The sinus node is a neomorphic development of specialized musculature in which the property of rhythmic discharge is highly developed. It is also a tissue of slow conductivity. With the discharge of the pacemaker in the node, the impulse spreads slowly over this node and then enters the auricular musculature by way of transitional muscle fibers. In the auricles it appears to travel faster and following the intricate branching of the auricular musculature ultimately stimulates, that is depolarizes, all parts of the auricles. Soon after depolarization, the depolarized auricular musculature repolarizes with a topographical spread more or less similar to that followed by depolarization so that the ordinary electrical record of

auricular activity shows two deflections, the P an T_A waves, opposite to each other.

In due course, the impulse in the auricles arrives at the auriculo-ventricular bridge described above and, passing through transitional fibers, it enters the auriculo-ventricular node. This node is also a neomorphic development of specialized muscle with anatomical and physiological properties resembling those of the sinus node. The claim that a special musculature bridge connects the two nodes (241, 242) appears to be unfounded except as a rare anomaly. Because the impulse travels slowly in nodal tissue, a delay in the transmission of the impulse to the ventricles is found. Once the impulse has passed the auriculo-ventricular node, it enters directly into the common auriculo-ventricular bundle, then its bundle branches and the terminal net-work of specialized muscular fibers. This latter system also is a neomorphic development of specialized muscle. In the common auriculo-ventricular bundle the character of the tissue quickly changes to the characteristic Purkinje fibers which are capable of rapid transmission of the impulse. It has been shown that except for the upper part of the interventricular septum, the terminal net-work penetrates all parts of the ventricular musculature (1, 2, 4). The bundle branches and the Purkinje net-work lie under the endocardium but the net-work also penetrates to the epicardium in all parts of the ventricles. The impulse therefore spreads simultaneously over many parts of the ventricles by means of this rapidly conducting terminal net-work, but in each locality arriving at the endocardium before reaching the epicardium. An objection to this view raised by Robb (263, 267, 271) has recently been withdrawn by her (264). In each part of the ventricles the impulse passes from the terminal Purkinje net-work via transitional fibers to the ventricular musculature proper. Thus the entire ventricular musclature is depolarized by a multitudinous series of quickly succeeding local impulses rapidly disseminated by the terminal net-work. This rapid spread makes the time of depolarization of the more massive ventricles of the same order of duration as that of the auricles, i.e., the QRS duration is of the same order as the P duration. Repolarization of the ventricles does not follow their depolarization as quickly as is the case in the auricles, so that a depolarized state expressed by the S-T segment is recorded in the ventricles. The electrical systole of the ventricles is much longer than that of the auricles. Repolarization of the ventricles does not follow the same topographic pattern as depolarization since the duration of electrical systole in the various parts of the ventricles shows considerable variation. Hence in many of the leads of the electrocardiogram the T wave is not opposite to the direction of the QRS deflection.

Many attempts have been made to depict the arrival of the impulse over the surface of the ventricles with various types of electrical connections in a number of species (2, 24, 88, 90, 91, 104, 123, 127, 128, 198, 199, 237, 257, 258, 271, 336, 343, 346). The data obtained with electrodes on various injured parts of the heart connected to a distant electrode can be dismissed as not suitable. Observations with the differential electrode, with electrodes on various uninjured parts of the heart connected to a distant electrode, and the more extensive data of

Harris (127, 128) with adjacent punctuate electrodes on various spots of the heart are more pertinent. The discrepancies in the observations of the various investigators can be explained on the basis of differences in the state of the exposed hearts studied and the adequacy of the criteria used to determine the arrival of the impulse locally. The results are in agreement, however, in showing 1, that stimulation of the external surface of the heart is not simultaneous and that the impulse often reaches regions far apart more nearly simultaneously than adjacent parts, and 2, that the total time occupied in stimulating the surface of the ventricles is much shorter than the duration of the QRS complex. The latter is partly due to the time occupied in stimulating the regions of the ventricles not bordering the external surface and partly due to the fact that the depolarization process in any region occupies time, which is significant when compared with the transmission time over the ventricles.

It is not feasible as yet, as some have attempted, to depict the exact details of the spread of the impulse through the auricles or ventricles. We are in ignorance of too many factors to be able to do this. However, some general comments on the direction of impulse spread in the average normal human heart can be made. In general in the auricles, the impulse spreads ventrally, caudally and to the left. This is adequately explained on the basis of the location of the sinus node in the upper right portion of the auricles and the assumption that there is a fairly uniform rate of spread through the auricles. There is no reason to assume the presence of specialized conducting paths or of synaptic-like junctions (256, 257, 258), nor is there need to assume that the impulse preferentially follows the spiral sheets and bundles of muscle.

In the case of the ventricles the situation is more complex. The impulse invades the septum before the outer walls of the ventricles. In the septum the muscle fibers proper appear to be reached first in two sites, one beneath the endocardium of the right and the other beneath that of the left ventricle, both sites being some distance below the auriculo-ventricular fibrous junction. The impulses from these two sites soon meet somewhere between the two endocardial surfaces. At the same time, the impulse front spreads radially through the septum parallel to its endocardial surfaces, in each region the subendocardium being involved earlier than the deeper layers. The impulse front reaches the auriculo-ventricular junction before the apex and interventricular grooves. After reaching the auriculo-ventricular junction the impulse front continues to spread toward the apex and at the same time toward the anterior and posterior interventricular grooves. On reaching the interventricular grooves the impulse front spreads over the lateral walls of the right and left ventricles. The front coming from the anterior interventricular groove and spreading in two directions meets that from the posterior interventricular groove, also spreading in two directions, about midway on the lateral surfaces of the right and left ventricles. The order of junction of the impulse fronts in the right and left ventricles is not predictable since the terminal net-work in the right heart travels obliquely from endocardium to epicardium while that of the left is nearly perpendicular to the endocardial surface (1), making up for the shorter distance across the thinner right ventri-

cular wall. However, it can be predicted as a general rule that the basal part of the lateral wall of both ventricles is stimulated last since the horizontal circumference of the heart is greatest at the base. Throughout, the subendocardium is stimulated ahead of the subepicardium and the papillary muscles appear to be stimulated ahead of adjacent muscle.

The Laws Governing the Spread of Current in a Volume Conductor. Some consideration must be given to the laws governing the spread of current in a volume conductor in order to appreciate the effect of the electric forces created in the heart upon the electrocardiogram recorded by electrodes located some distance from the heart. Space does not permit more than a superficial handling of this problem; the reader is referred to several excellent summaries for a more detailed and mathematical handling (13, 27, 61, 299, 314, 356, 367).

The simplest case of a volume conductor is that of a large finite electrically homogeneous sphere with the source of electric current at its center. If the source of current be considered a simple small cell which during its cycle of activity shows a wave of depolarization on its surface and then a wave of repolarization, the basic concepts can be expressed in simple terms. In the completely polarized resting state, it can be demonstrated that no potential differences exist in the outside conductor and no currents flow. Assuming the cell surface to be uniformly polarized, all points in the external conductor are at the same potential, namely zero. Some confusion has arisen in considering this problem because no clear distinction was made between the charge on the cell surface and its potential. Thus, according to the laws of spherical condensors it can be shown that the potential in the region outside of the cell and at its outside surface will be zero even though a positive charge is located on the outside of the cell surface (177, 316).

During depolarization, potential differences will be set up and currents will flow in the outside medium. While the actual source of the current is the polarized membrane remaining on parts of the cell surface, the effect can be accurately and more simply expressed by considering that the current is caused by a surface (or a series of surfaces) at the junction between the polarized and depolarized parts of the cell at any moment. The charge of this theoretical surface is oriented so that the positive charge faces the polarized part of the cell and the negative charge faces the depolarized part of the cell, and the intensity of the charge on the postulated surface is assumed to be identical with that on the actual polarized surface of the cell. A single imaginary surface will be adequate for the case where the process of depolarization at the moment is confined to a small region, i.e., that the rate of depolarization of any area is extremely high compared to the rate of propagation of the process to adjacent areas. If, however, the process of depolarization is relatively slower, then more accurate representation is obtained by assuming a series of planes located at the sites where each increment of depolarization is taking place. Thus, if the intensity of charge of the polarized membrane is considered to be N units, there would be N surfaces placed at the junctions where one unit of charge has been lost, each having a charge of one unit oriented as in the simpler case described above. Ordinarily in depolarization

little is lost if it is assumed that a single theoretical surface depicts the facts, since the series of surfaces which more accurately portray the facts would be very close to one another.

During repolarization, however, since repolarization of each region occupies much more time than does its depolarization, the series of theoretical surfaces would be farther apart and more error is introduced by reducing them to a single theoretical surface. Nevertheless this may still be done to simplify the solution provided one realizes the limitations of the method. When this is done, the single theoretical surface appears to have a smaller charge during repolarization than during depolarization. The orientation of the charges on the theoretical surface during repolarization is such that the positive charge faces the part of the cell already polarized and the negative charge faces that part still depolarized.

With the consideration of such imaginary surfaces, the potential of any point P in the outside medium would have the sign of the charge of the theoretical surface facing it. The magnitude of the potential at P would be a function of the intensity of the charge on the theoretical surface and of the solid angle subtended from the point P to the edge bounding the theoretical surface. Thus if the point P faced the side of the theoretical surface having a positive charge it would have a positive potential. And the intensity of the potential of the point P would increase 1, the closer it was to the theoretical surface; 2, the larger the rim of the theoretical surface; 3, the greater the intensity of charge of the theoretical surface; and 4, for points at equal distance from the center of the theoretical surface, the smaller the angle between the line connecting the point P to the center of the surface and the line from the center of the theoretical surface drawn perpendicular to it. In this fashion the lines of potential differences and the lines of current flow in the external field can be mapped out and the deflection produced in a galvanometer connected to any two points in the electric field predicted.

With some modifications these rules will apply also to the case where the size of the conducting medium is not so large and where it is not spherical.

This concept of theoretical surfaces serves to link the membrane theory with the facts observed in the case of the heart within the body. It explains the finding of a diphasic deflection with brusque transition during activation obtained when an electrode placed on the surface of the auricle is connected to one placed at a distance in the conducting medium. It is an improvement of Lewis' concept of "limited potential differences" (198) as developed by Craib (61) and most clearly enunciated by Wilson (367).

This view of a theoretical surface can also be applied to the case of injury currents. The theoretical surface of the resting injured cell would be at the junction between the injured and uninjured parts. The charge on the theoretical surface would be of a magnitude equal to the intensity of the charge on the uninjured region minus that on the injured part of the cell. The positive charge of the theoretical surface would face the uninjured part of the cell and the negative charge the injured part of the cell. During activity, if the injured part of the cell had retained a polarized state, the theoretical surface would remain but have its charge reversed and of different magnitude. During stimulation the lack

of response of the injured part of the cell, when such unresponsiveness existed, would alter the moment to moment orientation of the theoretical surface which would have existed if no injury were present; and during repolarization the effects of injury in retarding the repolarization of the injured part of the cell would also modify the moment to moment orientation of the theoretical surface which would have existed if no injury were present.

This concept of a theoretical surface will explain the findings 1, that an injury current is obtained when an electrode is placed on an injured part of the heart and connected with an electrode distant from the heart; 2, that this current can be reversed by moving the electrode on the heart to an uninjured region adjacent to the injury (80, 81, 89, 94, 156, 369), and 3, that this current decreases in magnitude rapidly when the electrode on the heart is moved farther away from the site of injury since the angle subtended by the postulated surface diminishes rapidly.

The theoretical surface concept makes it simpler to follow the events during the heart cycle and during injury since the charge giving rise to the electric field can be considered as located at the junction between depolarized and polarized parts of the cell, that is, at the junction between activated and restituted parts of the cell, and the junction between injured and uninjured parts of the cell.

The problem of following events during activation and restitution in the case of a complex cell like the auricles or ventricles can be simplified by this theoretical surface concept. Take the case of the auricles. Ordinarily the impulse spreads from a small area, from the sinus node, over the walls of the auricles caudad, ventrad and to the left, ignoring minor variations. The theoretical plane is therefore at right angles to this spread coinciding with the wave front, with the positive charge ahead of the negative. As it travels over the right auricle the length of the rim of this theoretical plane increases. Actually the wave front is a double ring, the inside ring on the subendocardial and the outer on the subepicardial aspect of the auricle and the theoretical surface being the surface between these rings approximately normal to the endocardial surface. Since it is assumed that the intensity of the polarized surface of the auricular syncytium is uniform, the increasing length of the rim of the theoretical surface will cause the potential differences to wax without much change in direction. As the impulse spreads over the left auricle and septum, the length of the rim will tend to decrease, causing the potential differences to wane. During this time, however, there will be a shift in the general direction of the impulse spread to the left rather than caudad. On the assumption that the topography of repolarization in the auricles follows closely that of the depolarization, the theoretical surface for repolarization will have the same characteristics as that during depolarization but 1, the charge will be reversed, and 2, the theoretical surface will have a less intense charge.

Take the case of the ventricles. As outlined earlier, two theoretical surfaces would start in the interventricular subendocardial surfaces and meet and fuse. During this time no potential differences would appear unless one of these impulses started ahead of the other, and even then the potential differences

created would not be large because the two theoretical surfaces would have oppositely directed charges. Apparently, under ordinary circumstances the stimulation via the left bundle precedes that via the right, making the theoretical plane entering the septum from the left larger than that from the right. After the two theoretical surfaces meet the further spread of the impulse would be radial in the septum, and an expanding narrow cylindrical theoretical surface would be present for a time. No potential differences would arise (except for the small differences explained above) until the cylinder front reached the auriculo-ventricular connective tissue partition. From that time on the narrow cylinder will be open at the top and the part facing toward the apex will therefore be unbalanced. Since it can be shown that regardless of how complex the theoretical surface actually is, it in turn is equivalent to the smallest surface encompassing its rim, such a new theoretical surface can be considered as representing the conditions at this time. In short, the potential field will be determined by this imagined secondary surface at the top of the septum, the positive charge of which faces the apex and the negative the auricles. This surface will expand until the entire junction of the septum with the auriculo-ventricular partition has been depolarized. The intensity of the potential differences will therefore grow. The field will be such as to have its positive potentials caudad, ventrad and to the left, that is, in the same general direction as in the field created during auricular stimulation.

As the impulse reaches and spreads through the lateral walls of the ventricles, some time before the whole base of the septum has been stimulated, the situation becomes more complex. The details have been briefly described above. Suffice it to say that the four wave fronts of impulse spread tend to neutralize one another except that any theoretical surface present on that part of the impulse front which moves toward the apex will be unopposed by any oppositely charged theoretical surface as soon as the wave front reaches the auriculo-ventricular junction. Should the base of the heart be completely activated before the apex then the field created would continue in general to be maintained in the direction found during stimulation of the septum, and will wax and wane. If, however, portions of the base of the heart are activated after the apex is completely stimulated, as is commonly the case, then the field will be markedly altered in its direction toward the end of the depolarization phase.

Injury of the heart situated in a conducting medium alters the electrical deflections obtained. During depolarization of the ventricles, the absence of response of the injured area leaves unbalanced certain theoretical surfaces which ordinarily would be counterbalanced by the response of the injured area, and so the QRS complex is altered. This is especially prominent when a precordial electrode is used over an injured area of the anterior surface of the heart. This alteration of QRS will persist when the area becomes necrotic or is replaced by scar tissue. In reality a new "window" is opened in the wall of the ventricle. Hence QRS changes as a result of injury occur equally readily whether the region is alive and injured, or dead, necrotic or scarred.

Both during the depolarized and repolarized state, the differences in polari-

zation between the injured and uninjured parts of the ventricles will set up a new theoretical surface giving rise to the injury current of rest and/or of activity. Its orientation will determine the potential differences in the field and so will determine the S-T deviation of injury. During repolarization the lag and protraction (or absence) of the repolarization process in the injured area will alter the characteristics of the theoretical surface at this time and set up the T wave alterations of injury.

Vector analysis. In a large finite spherical homogeneous conducting medium with the heart in the center, the electric forces could be resolved during the heart cycle into a series of successive vectors which alter in magnitude and direction. This is so since the electric forces have quantity, sign and direction, and can be added and resolved according to the law of vectors. It is in such a situation that an equilateral triangle can be created to calculate the manifest potential vectors in the frontal plane. In this situation also a tetrahedron composed of equilateral triangles, or a cube, can be created to calculate the actual three dimensional vectors.

Unfortunately the actual situation in the body is such that the attempts to determine the true vectors of the heart are extremely crude and give only a rough approximation of the momentary forces which exist in the heart (163). This is true for a number of reasons: 1, the torso of the body which constitutes the conducting medium is not spherical; 2, it is not a large field compared to the size of the heart (this can be appreciated by comparing the size of the fist to that of the torso); 3, the heart is not in the center but is closer to the sternum than the back, closer to the arms than to the legs and closer to the left than the right arm; 4, the limb leads used in obtaining the vectors in the frontal plane actually do not form an equilateral triangle (163), and 5, the body field is not composed of a homogeneous conducting medium (85, 167, 169, 173, 200), the lungs, for example, being poor conductors compared to the muscles of the back (169, 200). The view held by Wilson's laboratory that the electrical conductivity of the lungs is little different from other tissues is based on impedance measurements with extremely high frequency of alternating current (176). Such high frequency measurements are not applicable, since in electrocardiography we are concerned with fluctuating currents of much lower frequency. Actual experiments with insulation of various surfaces of the heart show clearly that the lungs are much poorer electrical conductors than blood, liver or muscle (169, 200). This gives certain parts of the heart a greater influence over others in creating the electric field of the body.

These discrepancies between conditions in the body and those postulated as ideal conditions for vector analysis are significant enough so that vector analysis such as has been attempted can give only a crude approach to the actual vectors existing during the heart cycle. Of course a rough idea of the direction and possible magnitude of the vectors can be obtained, but the approximation to the actual vectors remains to be established.

Another factor of importance is that the heart is so large that when electrodes are placed on certain parts of the body surface, as over the precordium, equal areas of the heart do not subtend the same angle at the electrode, consequently

they have unequal electric effects. Reduction of the electric forces to a single vector is inadequate since the part of the heart beneath the electrode plays a dominant rôle while more distant parts play a lesser rôle because the contribution of a given area of a polarized membrane to the potential is a function of the distance from the electrode and of the electrical conductivity of the intervening tissues. In this sense, and in this sense only, can the precordial electrode be considered a semidirect electrode. Under these circumstances it is difficult to see how a tetrahedron or cube can give more than a rough approximation of the true three dimensional vector. Attempts to get such stereovectograms are thus only rough approximations.

The construction of vectograms and stereovectograms (9, 27, 53, 54, 70, 74, 79, 135, 137, 149, 150, 151, 152, 154, 302, 303, 304, 305, 319, 320, 321,322,323, 328, 329, 351, 380, 381, 382, 383), the time course respectively of the vector in the frontal plane and in three dimensions, may give a crude picture of the summation of the electric forces existing in the heart from moment to moment during the heart cycle, and can help to approximate the character of the forces in various circumstances. On a purely empiric basis they may prove to be of diagnostic value in various circumstances, but they have not yet been found to serve this purpose. Even if this were accomplished, it cannot be considered that they actually depict the true summation of the electric forces of the heart cycle except in a distorted fashion.

The attempt to summate the vectors during depolarization, during repolarization and during the entire ventricular electrical systole, the resultant vectors being known respectively as the QRS vector, the T (or more properly the S-T-T) vector and the ventricular gradient; (11, 12, 15, 16, 17, 18, 19, 20, 97, 158, 212, 213, 354, 368) may also prove ultimately to be of practical value. However, these summations introduce other errors which may become misleading. For example, the QRS vector of the frontal plane may give an identical value in the case of a deep Q_2 and Q_3 (i.e., a deep Q in leads 2 and 3) as in the case of a deep S_2 and S_3 and yet the frontal vectogram (i.e., a graph of the instantaneous vectors throughout the heart cycle) will be entirely different under these two circumstances. Again the S-T-T vector caused by an inverted coronary T_2 and T_3 and an elevated S-T_2 and S-T_3 may be identical with that obtained when T_2 and T_3 are upright and S-T_2 and S-T_3 are depressed, and yet the frontal vectogram will be entirely different in the two cases. Such summated vectors may thus conceal significant differences and the ventricular gradient derived from them may also be non-revealing. Of course, it is true that the measurements of the ventricular gradient can help to estimate whether a change in the S-T segment and T wave are due to positional alterations of the heart on the one hand, or to injury, heart strain or some influence selectively affecting the repolarization process on the other. In our experience, however, examination of the contour of the electrocardiogram in the limb leads and chest leads will serve this function as well if not better (164). The latter method will in fact avoid the implication that the solution is a mathematical one and therefore necessarily the correct one.

It can be seen from the discussion above that there are two opposing viewpoints.

One overlooks individual variability and seeks a simple mathematical solution. The other is perturbed by the inaccuracies of the mathematical approach and fears that by following it important variables may be neglected. There is merit in both approaches provided zealots do not overestimate or underestimate the relative merits of the opposing viewpoints. Considerable work remains to be done in defining the limits of both points of view. It is as much an error to deny the possibility that the true electric forces of the heart can be reconstructed from the electrocardiogram as it is to affirm that at the present time the estimated vector is adequate, fairly accurate and sufficiently informative for ordinary use.

So-called unipolar leads. Another experimental approach remains to be discussed and that is the attempt to obtain the actual potentials of single points in the conducting medium (25, 52, 55, 111, 112, 113, 114, 115, 129, 179, 219, 220, 355, 356). For practical purposes there is little to be gained from these attempts but the problem of a suitable method to accomplish this is intriguing. If one were dealing with an infinite sphere of a homogeneous conducting medium the potentials of single points might be measurable but with the situation as it exists in the human body this is not strictly possible although approximations may be obtained. The use of the central terminal connected to the three extremities through 5000 ohms each has been widely discussed (cf. 356). Even the proponents who introduced this method recognize that while the central terminal may show less fluctuation in potential than any single distant electrode, it need not be at constant zero potential (317, 356). For example, it can happen that all three limb electrodes may some time during the heart cycle be in the negative field while the positive field is concentrated around the anterior precordium or vice versa. Under these circumstances the central terminal will also have a negative potential (or a positive one) greater than that of one or more electrodes to which it is connected. During this time it will be a "worse" contact for obtaining the true potential of the exploring electrode than some other single electrode. Goldberger's so-called augmented unipolar lead (111, 112, 113, 114, 115) fails on theoretical grounds to meet even the requirements set up for the Wilson terminal, especially when it is connected to only two of the three limb electrodes. Other attempts to obtain the true potential of the exploring electrode have been made. One procedure is to immerse the body in a metal lined tub filled with a strong salt solution (51, 52, 78, 219), the patient breathing by way of a tube passed up through the bath. The entire metal lining of the tub is used as the indifferent null electrode. This is probably as close to a null electrode as one can obtain, but is not too practical. A variant of this procedure is to employ a broad metal covering of a large part of the body which should approach a null electrode more nearly than the central terminal of Wilson (179, 220). However, it would appear that an absolute null electrode has not yet been perfected.

Great care must likewise be employed in using precordial leads to determine the time of arrival of the impulse beneath the chest electrode (356, 357, 364) since distant effects obscure the so-called intrinsic deflection especially when the electrode is not placed over the region of solid dullness.

The Influence Exerted by the Characteristics of the Body as a Conductor of Electri-

city. In the last section it was pointed out that the body differs from the ideal spherical conducting medium of large finite size and that on this account variables are introduced which make accurate calculation of the vectors impossible. These characteristics of the body as a conducting medium also affect the character of the electrocardiogram. Their variation in different individuals and in disease affect electrocardiographic contour. Some of these qualities have unimportant, others more striking effects.

Persons vary in the relative diameters of their torsos. The body field of a fat person is different from that of a thin individual. It is doubtful, however, that these changes in body shape have any significant influence on the electrocardiogram except as they are accompanied by rotation of the heart.

More significant and commonly overlooked is the effect of variations in the electricial conductivity of the medium adjacent to the heart. For example, in emphysema where the lungs, abnormally distended by air, encroach upon the heart and increase the insulation around it, the potential differences set up in the external field tend to be smaller and current flow would largely be confined to the heart and pericardium. As a result, the amplitude of the deflections in the electrocardiogram tends to become smaller. Likewise pericardial or pleural effusions lying close to the heart are good electrical conductors and tend to shunt the external field which is of higher resistance, thereby decreasing the current flow through the body field. Hence, here too, the amplitude of the deflections of the electrocardiogram decreases. The presence of focal pleural effusions or of consolidations in the lungs near the heart tend to produce changes in the electric field of the body and to modify the electrocardiographic contour. In short, alterations of the character of the electrical conductors adjacent to the heart modify the field created and thereby alter the recorded electrical deflections (163). Such changes could affect the magnitude of the vectors reconstructed from the electrocardiogram, without affecting the orientation of the electric forces within the heart.

The Variations Introduced by Modification of the Anatomy and Physiology of the Heart. Variations in the anatomy and physiology of the heart are far more important than variations in the character of the body field in determining electrocardiographic contour.

1. Variations in the normal heart. The recent experience of the war when electrocardiograms on large numbers of normal persons were taken has clearly shown the wide range of variation of the "normal" electrocardiogram caused by differences between individuals. Considerable variation is also found in the same individual in the course of ageing. These variations with age determine the different normal limits of the electrocardiogram in infants, children and adults. The details of the anatomic and physiologic differences of normal hearts in the various age groups will not be considered here (cf. 164, 172, 282). They depend, as do the variations in normal contour in any age group, on 1, the shape and size of the heart; 2, the position of its various axes in relation to the body, and 3, variations in the conduction paths for the impulse and in the location of the impulse origin.

The shape of the heart and the relative size of its various chambers show some variation. This is particularly striking when one compares the heart of an infant with that of an adult. But even in the adult there is a considerable range in shape of the heart. Take for example the heart in which the diameter from base to apex is longer than the other two diameters and compare it with a heart in which the reverse is true. In the first case, assuming uniform rate of impulse spread, the arrival of the impulse at the apex of the septum will be delayed as compared with its arrival near the base of the anterior and posterior interventricular grooves. In the second case, the impulse will reach the apex sooner than the basal parts of the lateral walls of the ventricles at the interventricular grooves. Obviously the topography of the spread of the impulse will be sufficiently different in the two cases to vary the QRS configuration. One could write as extensive a treatise on the effects of such and other differences in the shape of the heart on the QRS vector as Ashman has on the effect of normal changes in the heart's position (97).

The subject of variations in the normal position of the heart has been covered extensively elsewhere (5, 6, 14, 97, 357) and need not be recapitulated here. It is apparent that this is of importance in accounting for the wide range in contour of the various deflections of the normal electrocardiogram.

A similar effect can be demonstrated for variations in the anatomy and function of the specialized muscle of the heart from which the impulse originates and over which it spreads. Changes in the P wave may come about not only because of altered shape of the auricles or alterations in the position of their axes relative to the body but also because the pacemaker may be located in the body or tail of the sinus node instead of the head. Similar P wave contour changes may occur when the site at which the impulse leaves the sinus node is different from the usual point of exit, even though the point of its origin has not shifted.

The anatomy of the specialized conducting system in the ventricles shows a normal range of variation from one heart to another, and these variations together with the variations in the speed of impulse transmission through the various elements of the bundle branch system and the associated terminal Purkinje net-work participate in the production of variations in the configuration of the ventricular deflections in the electrocardiogram.

Not enough attention has been paid to the factors just discussed, namely, the variations in the normal anatomy and physiology of impulse origin and spread, the normal variations in size and shape of the heart and the normal variations in the location and properties of the electric conductors adjacent to the heart in comparison to the extensive consideration given to the normal variations in the location of the axes of the heart. As a result, the latter have been given undue emphasis and some workers have resorted to nebulous terms like "electrocardiographic vertical position of the heart" to explain certain patterns of the electrocardiogram not revealed by fluoroscopic or x-ray examinations of the heart.

2. Variations in the abnormal heart. Changes in shape, size and position of the heart play an important rôle in determining the electrocardiographic contour in the abnormal heart (184). Dilatation of the various chambers, displacements of

the entire heart and rotation on the various axes of the heart are common in disease and play a significant rôle in determining the appearance of the electrocardiogram. These factors have only recently been given adequate consideration in evaluating abnormalities of the electrocardiogram.

Heart disease also produces electrocardiographic alterations by causing hypertrophy of the various chambers leading thereby to heart strain patterns. These come about because of the prolongation and altered manner of impulse spread in the hypertrophied chamber which prolongs the time of depolarization to a slight degree, alters the balance of electric forces during this time and tends to make the pattern of repolarization follow more nearly the pattern of depolarization in time and space than is normally the case.

Other pathological changes in electrocardiographic contour are caused by regions of ischemia and injury. By leading to unresponsive areas, to injury currents of rest, of activity and/or of repolarization, they are responsible for many modifications of the electrocardiogram. Sometimes these are sufficiently striking so as to constitute specific patterns easily recognizable in the electrocardiogram.

Disease may modify the electrocardiogram by altering the speed of repolarization in the whole heart or in different parts of the heart. Finally, disease leads to abnormalities in electrocardiographic contour by displacing the location of impulse origin and by producing delays or blockage in various parts of the path over which the impulse spreads.

Drugs operate in fashions similar to disease in causing electrocardiographic alterations.

It is the task of the electrocardiographer on the basis of experience and some knowledge of the theory of the genesis of the electrocardiogram to interpret properly the abnormalities in its contour. How this is accomplished has been considered at length elsewhere (164). A major aim of these efforts to unravel the genesis of the electric forces of the heart must be the advancement of the ability to properly evaluate the electrocardiogram for clinical utility.

SUMMARY

The present review concerns itself with the theoretical aspects of the electrocardiogram. Current theories and findings are critically evaluated from the author's viewpoint.

The subject is developed under the following four headings:

a. The genesis and typical time course of the electric states of the heart during activity and injury.

b. The laws governing the spread of current within a volume conductor.

c. The influence exerted by the characteristics of the body as a conductor of electricity.

d. The modifications introduced by variations of the anatomy and physiology of the heart.

a. The genesis of the electric states of the heart is discussed in terms of the classical hypothesis of polarization, depolarization and repolarization of the cell

surface corresponding to rest, activity and recovery, respectively. Modern refinements of these concepts do not significantly modify the classical concepts of the production of the main deflections recorded in the electrocardiogram. Alterations of the normal record caused by regional injuries to the heart muscle are explained and classified according to the mode of action of injury; injuries may be such as to produce changes during rest, activity or the recovery phase, or lead to a combination of these. The present state of the problem of neurogenic versus myogenic impulse spread and of the syncytial character of heart muscle is discussed. The view is expressed and defended that, some recent evidence to the contrary, myogenic transmission accounts for the spread of the impulse, and that, on the basis of their syncytial character, the auricles and the ventricles each acts electrically as a single cell. A brief detailed topographical and chronological account is presented of the spread of the impulse as the basis on which the typical time sequence of the deflections seen in the electrocardiogram can be understood.

b. A discussion follows of the modification introduced into the recording of the electric states of the heart produced by the circumstance that the recording electrodes are placed at a distance from the source of current. These modifications are considered in terms of theoretical charged surfaces interposed between polarized and depolarized regions of the heart which alter in position and direction of charge as the sources and sinks of current shift during the heart cycle. The influence of injury upon such theoretical charged surfaces is also considered. Next, the attempt to summate the electric forces created during the heart cycle in terms of vector analysis is presented. Its main limitation is seen in the fact that the initial assumptions from which the analysis proceeds are, as yet, crude oversimplifications which must lead to a distorted picture of events in the heart.

c. Aside from the distortions generally introduced by considering the body as a large sphere of homogeneous conducting properties with the heart at its center, individual distortion may be introduced by pathological variation of the conducting media next to the heart.

d. Considerably more importance is attached to the effect of modifications in the anatomy and physiology of the heart itself in altering the contour and time pattern of the normal electrocardiogram. As examples of such modifications of the normal heart are discussed differences in size and shape between the infantile and the adult heart, the effect of variations in the relative length of the two main heart diameters from one heart to another, and the well worked out effect of variations in the position of the main axes of the heart with respect to the body. Of even more striking effect than these normal variations are, of course, pathological variations in the anatomy and physiology of the heart which are encountered in disease.

REFERENCES

(Essentially the significant literature since 1930)

(1) Abramson, D. I. and J. C. Cardwell. A new anatomic basis for the spread of the impulse in the mammalian ventricle. Am. J. Physiol. 109: 1, 1934.

(2) ABRAMSON, D. I. AND K. JOCHIM. The spread of the impulse in the mammalian ventricle. Am. J. Physiol. **120:** 635, 1937.

(3) ABRAMSON, D. I., L. N. KATZ, S. MARGOLIN AND S. LOURIE. Variations in the electrocardiographic form of experimental ventricular ectopic beats induced in the monkey and dog. Am. Heart J. **13:** 217, 1937.

(4) ABRAMSON, D. I. AND S. MARGOLIN. A Purkinje conduction network in the myocardium of the mammalian ventricles. J. Anat. **70:** 250, 1936.

(5) ABRAMSON, D. I. AND J. WEINSTEIN. A basis for the analysis of variations in the form of electrocardiographic curves resulting from experimental premature contractions. Am. J. Physiol. **115:** 569, 1936.

(6) ACKERMAN, W. AND L. N. KATZ. Reversal in direction of the QRS complex of experimental right bundle branch block with change in the heart's position. Am. Heart J. **8:** 490, 1933.

(7) AMUCHASTEGUI, S. R. O., O. ORIAS AND A. S. SEGURA. Variaciones de los complejos inicial y final de electrogramas miocardicos segun la dirección de propagación del estímulo. Rev. Soc. Argent. Biol. **18:** 138, 1942.

(8) ARMSTRONG, P. B. Functional reactions in the embryonic heart accompanying the ingrowth and development of the vagus innervation. J. Exper. Zool. **58:** 43, 1931.

(9) ARRIGHI, F. P. El eje eléctrico del corazón en el espacio. Prensa Med. Argent. **26:** 253, 1939.

(10) ASHMAN, R. An outline of electrocardiography. New International Clinics **1:** 193, 1939.

(11) ASHMAN, R. The normal human ventricular gradient. IV. The relationship between the magnitudes, AQRS and G, and deviations of the RS-T segment. Am. Heart J. **26:** 495, 1943.

(11a) ASHMAN, R. Heart. Ann. Rev. Physiol. **6:** 319, 1944.

(12) ASHMAN, R. A statistical study of the ventricular gradient and of the QRS complex of the electrocardiogram. Arch. Inst. Cardiología México **15:** 266, 1945.

(13) ASHMAN, R. Nociones fundamentales de electrocardiografía. Rev. Argent. Cardiol. **12:** 220, 1945.

(14) ASHMAN, R. Estimation of heart position from the QRS complex of the electrocardiogram. Arch. Inst. Cardiología México **16:** 139, 1946.

(15) ASHMAN, R., E. BYER AND R. H. BAYLEY. The normal human ventricular gradient. I. Factors which affect its direction and its relation to the mean QRS axis. Am. Heart J. **25:** 16, 1943.

(16) ASHMAN, R. AND E. BYER. The normal human ventricular gradient. II. Factors which affect its manifest area and its relationship to the manifest area of the QRS complex. Am. Heart J. **25:** 36, 1943.

(17) ASHMAN, R., F. P. FERGUSON, A. I. GREMILLION AND E. BYER. The effect of cycle-length changes upon the form and amplitude of the T deflection of the electrocardiogram. Am. J. Physiol. **143:** 453, 1945.

(18) ASHMAN, R., F. P. FERGUSON, A. I. GREMILLION AND E. BYER. The normal human ventricular gradient. Am. Heart J. **29:** 697, 1945.

(19) ASHMAN, R, F. P. FERGUSON, A. I. GREMILLION AND E. BYER. Effect of cardiac cycle-length upon magnitude of ventricular gradient. Proc. Soc. Exper. Biol. and Med. **59:** 47, 1945.

(20) ASHMAN, R., M. GARDBERG AND E. BYER. The normal human ventricular gradient. III. The relation between the anatomic and electrical axes. Am. Heart J. **26:** 473, 1943.

(21) ASHMAN, R. AND R. HAFKESBRING. Unidirectional block in heart muscle. Am. J. Physiol. **91:** 65, 1929.

(22) ASHMAN, R., W. S. WILDE AND C. E. DRAWE. The validity of the electrical doublet theory of the cardiac action current. Am. J. Physiol. **128:** 547, 1940.

(23) ASHMAN, R. AND N. C. WOODY. Monophasic action currents from the uninjured turtle ventricle. Proc. Soc. Exper. Biol. and Med. **42:** 17, 1939.

(24) Barker, P. S., A. G. Macleod and J. Alexander. The excitatory process observed in the exposed human heart. Am. Heart J. **5:** 720, 1930.

(25) Barker, P. S., F. N. Wilson and F. D. Johnston. Electrocardiograms that record the potential variations produced by the heart beat at a single point. J. Clin. Investigation **12:** 969, 1933.

(26) Bayley, R. H. The potential produced by cardiac muscle. A general and a particular solution. Proc. Soc. Exper. Biol. and Med. **42:** 699, 1939.

(27) Bayley, R. H. On certain applications of modern electrocardiographic theory to the interpretation of electrocardiograms which indicate myocardial disease. Am. Heart J. **26:** 769, 1943.

(28) Bayley, R. H. The electrocardiographic effects of injury at the endocardial surface of the left ventricle. Am. Heart J. **31:** 677, 1946.

(29) Bayley, R. H. and J. S. La Due. Electrocardiographic changes of impending infarction. Am. Heart. J. **28:** 54, 1944.

(30) Bayley, R. H. and J. S. La Due. Differentiation of the electrocardiographic changes produced in the dog by prolonged temporary occlusion of a coronary artery from those produced by postoperative pericarditis. Am. Heart J. **28:** 233, 1944.

(31) Bayley, R. H., J. S. La Due and D. J. York. Electrocardiographic changes (local ventricular ischemia and injury) produced in the dog by temporary occlusion of a coronary artery, showing a new stage in the evolution of myocardial infarction. Am. Heart J. **27:** 164, 1944.

(32) Bayley, R. H., J. S. La Due and D. J. York. Further observations on the ischemia-injury pattern produced in the dog by temporary occlusion of a coronary artery. Am. Heart J. **27:** 657, 1944.

(33) Benjamin, J. E., H. Landt and L. R. Culver. The body as a volume conductor and its influence on the electrical field of the heart. Am. J. Med. Sci. **195:** 759, 1938.

(34) Bishop, G. H. La théorie des circuits locaux permet-elle de prévoir la forme du potentiel d'action? Arch. Intern. Physiol. **45:** 273, 1937.

(35) Bishop, G. H. The relation of bioelectric potentials to cell functioning. Ann. Rev. Physiol. **3:** 1, 1941.

(36) Bishop, G. H. and A. S. Gilson. Action potentials from skeletal muscles. Am. J. Physiol. **89:** 135, 1929.

(37) Blair, D. M. and F. Davies. Observations on the conducting system of the heart. J. Anat. **69:** 303, 1935.

(38) Blair, D. M., F. Davies and E. T. B. Francis. Conducting system of marsupial heart. Trans. Roy. Soc. Edinburgh **60:** 629, 1943.

(39) Blair, H. A., A. M. Wedd and A. C. Young. The relation of the Q-T interval to the refractory period, the diastolic interval, the duration of contraction and the rate of beating in heart muscle. Am. J. Physiol. **132:** 157, 1941.

(40) Bogue, J. Y. The electrocardiogram of the developing chick. J. Exper. Biol. **10:** 286, 1933.

(41) Bohning, A., L. N. Katz and R. Langendorf. The distribution of surface potential on the chest in intraventricular block. Am. Heart J. **22:** 778, 1941.

(42) Bohning, A., L. N. Katz, R. Langendorf and B. Blumenthal. Intraventricular block, including so-called bundle branch block. Am. J. Med. Sci. **202:** 671, 1941.

(43) Bohning, A., L. N. Katz, M. Robinow and G. Gertz. The value and significance of multiple chest leads in man. I. Normal and hypertrophied hearts. Am. Heart J. **18:** 25, 1939.

(44) Boyd, L. J. and D. Scherf. The electrocardiogram after mechanical injury of the inner surface of the heart. Bull. N. Y. Med. College **3:** 1, 1940.

(45) Bozler, E. The initiation of impulses in cardiac muscle. Am. J. Physiol. **138:** 273, 1943.

(46) Brown, W. H. A study of the esophageal lead in clinical electrocardiography. I. Am. Heart. J. **12:** 1, 1936.

(47) Brown, W. H. A study of the esophageal lead in clinical electrocardiography. II. Am. Heart J. **12:** 307, 1936.

(48) Buchbinder, W. and L. N. Katz. The electrocardiogram in acute experimental distention of the right heart. Am. J. Med. Sci. **187:** 785, 1934.

(49) Büchner, F. Die Deutung des Elektrokardiogramms bei den Durchblutungsstörungen des Herzmuskels. Klin. Wchnschr. **17:** 1713, 1938.

(50) Burge, W. E., O. S. Orth, H. W. Neild, R. Krouse and G. C. Wickwire. Cause and significance of electronegativity of active living tissue. J. Lab. Clin. Med. **21:** 1162, 1936.

(51) Burger, R. Über das elektrische Feld des Herzens; über die herzferne Electrode bei der semidirekten Ableitung; über die Nullpotentialelektrode von Wilson; über die Verwendung einer von der gesamten Körperoberfläche ableitenden Elektrode als indifferente Test-Elektrode. Cardiologia **3:** 56, 1939.

(52) Burger, R. and F. Wuhrman. Über das elektrische Feld des Herzens. II. Cardiologia **3:** 139, 1939.

(53) Burger, R. and F. Wuhrman. Über elektrokardiographische Analyse mittles Vektordiagramm und elektrischem Feld; Demonstration an einem Vorderwand-Spitzen-Infarkt mit W-form des Initialkomplexes in den Ableitungen I and II und kleinen Ausschlägen in allen Extremitäten-Ableitungen. Schweiz. med. Wchnschr. **71:** 65, 1941.

(54) Burger, R. and F. Wuhrman. Der Erregungsablauf im Herzen und das elektrische Feld, demonstriert an einem Fall von sogenanntem Wilson-Block. Schweiz. med. Wchnschr. **71:** 261, 1941.

(55) Cabrera, E. and D. Sodi Pallares. Determinación del eje medio manifiesto por las derivaciones unipolares de los miembros. Arch. del Inst. Cardiologica México **14:** 135, 1945.

(56) Calabresi, M. and A. J. Geiger. Potential changes in injured cardiac muscle. Am. J. Physiol. **137:** 440, 1942.

(57) Canfield, R. On the electrical field surrounding doublets and its significance from the standpoint of Einthoven's equations. Heart **14:** 102, 1927.

(58) Cardwell, J. C. and D. I. Abramson. The atrioventricular conduction system of the beef heart. Am. J. Anat. **49:** 167, 1931.

(59) Cole, K. S. and H. J. Curtis. Electrical impedance of Nitella during activity. J. Gen. Physiol. **22:** 37, 1938.

(59a) Cole, K. S. and R. F. Baker. Longitudinal impedance of the squid giant axon. J. Gen. Physiol. **24:** 771, 1940–41.

(60) Correa, R. M. E. Citología del miocardio y sistema de conducción aurículo-ventricular (en el perro). Rev. Soc. Argent. Biol. **14:** 486, 1938.

(61) Craib, W. H. The electrocardiogram. Privy Council, Med. Research Council, Spec. Report, Series no. 147, 1930.

(62) Curtis, H. J. and K. S. Cole. Membrane action potentials from the squid giant axon. J. Cell. Comp. Physiol. **15:** 147, 1940.

(63) Curtis, H. J. and K. S. Cole. Membrane resting and action potentials of the squid giant axon. Am. J. Physiol. **133:** 254, 1941.

(64) Davies, F. The conducting system of the bird's heart. J. Anat. **64:** 129, 1930.

(65) Davies, F. Further studies of the conducting system of the bird's heart. J. Anat. **64:** 319, 1930.

(66) Davies, F. The conducting system of the monotreme heart. J. Anat. **65:** 339, 1931.

(67) Davies, F. The conducting system of the vertebrate heart. Brit. Heart J. **4:** 66, 1942.

(68) Davies, F. and E. T. B. Francis. The heart of the salamander with special reference to the conducting (connecting) system and its bearing on the phylogeny of the conducting systems of mammalian and avian hearts. Phil. Trans. Royal Soc. London Series B., **231:** 99, 1941.

(69) De Boer, S. Researches on the electrocardiogram. Cardiologia **2:** 292, 1938.
(70) Decherd, G. M. and A. Ruskin. Momentary atrial electrical axes. I. Normal sinus rhythm. Am. Heart J. **28:** 794, 1944.
(71) Decker, M. Über den Anstieg der Aktionsspannungskurve des Ventrikels. Arch. f. Kreislauf. **6:** 75, 1940.
(72) Donnan, F. G. The theory of membrane equilibria. Chemical Rev. **1:** 73, 1924.
(73) Drury, A. N. and D. W. Mackenzie. The influence of vagal stimulation upon conduction through the branches of the A-V bundle in the dog. J. Physiol. **80:** 329, 1934.
(74) Duchosal, P. W. Physiopathologie de l'electrocardiogramme. Helv. Med. Acta. **12:** 361, 1945.
(75) Eccles, J. C. and H. E. Hoff. The rhythm of the heart beat. I. Location, action potential and electrical excitability of the pacemaker. Proc. Roy. Soc. B. **115:** 307, 1934.
(76) Eccles, J. C. and H. E. Hoff. The rhythm of the heart beat. II. Disturbance of rhythm produced by late premature beats. Proc. Roy. Soc. B. **115:** 327, 1934.
(77) Eccles, J. C. and H. E. Hoff. The rhythm of the heart beat. III. Disturbances of rhythm produced by early premature beats. Proc. Roy. Soc. B. **115:** 352, 1934.
(78) Eckey, P. and R. Fröhlich. Zur Frage der unipolaren Ableitung des Elektrokardiogramms. Arch. f. Kreislauf. **2:** 349, 1938.
(79) Einthoven, W., G. Fahr and A. de Waart. Über die Richtung und die manifeste Grösse der Potentialschwankungen im menschlichen Herzen und über den Einfluss der Herzlage auf die Form des Elektrokardiogramms. Pflüger's Arch. **150:** 308, 1913.
(80) Eyster, J. A. E. and W. E. Gilson. The development and contour of cardiac injury potential. Am. J. Physiol. **145:** 507, 1946.
(81) Eyster, J. A. E. and H. Goldberg. The relation of injury potentials in heart muscle to other electrical and to mechanical events. Am. J. Physiol. **133:** 272, 1941.
(82) Eyster, J. A. E., M. R. Krasno and J. P. Hettwer. Electrical potentials of the heart of the chick embryo. Am. J. Physiol. **120:** 173, 1937.
(83) Eyster, J. A. E., M. R. Krasno, C. A. Maaske and M. J. Ulevich. The origin of the R and T potentials from the mammalian heart. Am. J. Physiol. **120:** 663, 1937.
(84) Eyster, J. A. E., F. Maresh and M. R. Krasno. The electric field around heart muscle. Am. J. Physiol. **105:** 33, 1933.
(85) Eyster, J. A. E., F. Maresh and M. R. Krasno. The nature of the electrical field around the heart. Am. J. Physiol. **106:** 574, 1933.
(86) Eyster, J. A. E., F. Maresh and M. R. Krasno. The electrical field around heart and skeletal muscle. Am. J. Physiol. **109:** 34, 1934.
(87) Eyster, J. A. E., F. Maresh and M. R. Krasno. The nature of the R wave potentials in the tortoise and frog heart. Am. J. Physiol. **110:** 422, 1934.
(88) Eyster, J. A. E. and W. J. Meek. The sequence of fractionate contraction at different surface regions on the right auricle and ventricles of the dog's heart. Am. J. Physiol. **134:** 513, 1941.
(89) Eyster, J. A. E. and W. J. Meek. Cardiac injury potentials. Am. J. Physiol. **138:** 166, 1942.
(90) Eyster, J. A. E., W. J. Meek and W. E. Gilson. Spread of impulse over the ventricles in the mammalian heart. Am. J. Physiol. **123:** 61, 1938.
(91) Eyster, J. A. E., W. J. Meek and H. Goldberg. The relation of the electrical and mechanical events in the dog's heart and the spread of activity in the right auricle and both ventricles. Am. J. Physiol. **129:** 354, 1940.
(92) Eyster, J. A. E., W. J. Meek and H. Goldberg. The relation between electrical and mechanical events in the dog's heart. Am. J. Physiol. **131:** 760, 1941.

(93) Eyster, J. A. E., W. J. Meek, H. Goldberg and H. L. Bartsch. The sequence of onset of injury potentials on the surface of the dog and turtle ventricle. Am. J. Physiol. **126:** 488, 1939.

(94) Eyster, J. A. E., W. J. Meek, H. Goldberg and W. E. Gilson. Potential changes in an injured region of cardiac muscle. Am. J. Physiol. **124:** 717, 1938.

(95) Fahr, G. and A. Weber. Über die Ortsbestimmung der Erregung in menschlichen Herzen mit Hilfe der Elektrokardiographie. Deutsch. Arch. f. klin. Med. **117:** 361, 1915.

(96) Feil, H. S., L. N. Katz, R. A. Moore and R. W. Scott. The electrocardiographic changes in myocardial ischemia. Am. Heart J. **6:** 522, 1931.

(97) Gardberg, M. and R. Ashman. The QRS complex of the electrocardiogram. Arch. Int. Med. **72:** 210, 1943.

(98) Garrey, W. E. and C. E. King. Impulse transmission in the sinus venosus of the turtle heart. Am. J. Physiol. **133:** 288, 1941.

(99) Garrey, W. E. and C. E. King. Localization of the pacemaker in the sinus venosus of the turtle heart. Am. J. Physiol. **133:** 288, 1941.

(100) Géraudel, E. La théorie vestibulaire du mécanisme cardiaque. Presse Méd. **43:** 297, 1935.

(101) Gilson, A. S. Factors determining block of the conducted cardiac impulse. Am. J. Physiol. **110:** 376, 1935.

(102) Gilson, A. S. and G. H. Bishop. The interpretation of electrocardiograms obtained from heart strips in a conducting medium. Am. J. Physiol. **109:** 40, 1934.

(103) Gilson, A. S. and G. H. Bishop. The effect of remote leads upon the form of the recorded electrocardiogram. Am. J. Physiol. **118:** 743, 1937.

(104) Gilson, W. E., R. E. Conklin and J. A. E. Eyster. The relation of fractionate contraction at various surface regions of the ventricles of the snapping turtle to the surface potential distribution. Fed. Proc. **1:** 29, 1942.

(105) Glomset, D. J. and R. F. Birge. A morphologic study of the cardiac conduction system. IV. The anatomy of the upper part of the ventricular septum in man. Am. Heart J. **29:** 526, 1945.

(106) Glomset, D. J. and A. T. A. Glomset. A morphologic study of the cardiac conduction system in ungulates, dog and man. I. The sino-atrial node. Am. Heart J. **20:** 389, 1940.

(107) Glomset, D. J. and A. T. A. Glomset. A morphologic study of the cardiac conduction system in ungulates, dog and man. II. The Purkinje system. Am. Heart J. **20:** 677, 1940.

(108) Glomset, D. J., A. T. A. Glomset, B. D. Glomset and R. F. Birge. A morphologic study of the cardiac condution system. III. Bundle branch block. Am. Heart J. **28:** 348, 1944.

(109) Goldberg, H. and J. A. E. Eyster. The electrical field on the surface of the active turtle ventricle. Am. J. Physiol. **126:** 503, 1939.

(110) Goldberg, H. and J. A. E. Eyster. The relation of electrical and mechanical events in the ventricle of the turtle. Am. J. Physiol. **128:** 390, 1940.

(111) Goldberger, E. A simple, indifferent, electrocardiographic electrode of zero potential and a technique of obtaining augmented, unipolar, extremity leads. Am. Heart J. **23:** 483, 1942.

(112) Goldberger, E. The aVl, aVr, and aVf leads; simplification of a standard lead electrocardiographically. Am. Heart J. **24:** 378, 1942.

(113) Goldberger, E. Use and advantages of augmented unipolar extremity leads in electrocardiographic diagnosis of myocardial infarction. N. Y. State J. Med. **43:** 961, 1943

(114) Goldberger, E. Advantages of augmented unípolar extremity leads (aV leads) over the standard leads in clinical electrocardiography. Rev. Argent. Cardiol. **11:** 149, 1944.

(115) GOLDBERGER, E. Studies on unipolar leads. IV. Effects of digitalis. Am. Heart J. **28:** 370, 1944.

(116) GOLDBERGER, E. An interpretation of axis deviation and ventricular hypertrophy. Am. Heart J. **28:** 621, 1944.

(117) GOLDBERGER, E. The validity of the Einthoven triangle hypothesis. Am. Heart J. **29:** 369, 1945.

(118) GOLDBERGER, E. The differentiation of normal from abnormal Q waves. Am. Heart J. **30:** 341, 1945.

(119) GOLDBERGER, E. Significance of downward T waves in precordial leads of normal children. Am. J. Dis. Children **71:** 618, 1946.

(120) GOLDENBERG, M. AND C. J. ROTHBERGER. Über das Elektrogramm der spezifischen Herzmuskulature. Pflüger's Arch. **237:** 295, 1936.

(121) GROEDEL, F. M. How far does the situation of the indifferent electrode influence the electrocardiographic unipolar chest leads. Cardiologia **3:** 23, 1938.

(122) GROEDEL, F. M. Topography and time of appearance of the action-potential of the heart on the anterior and posterior chest wall in young healthy persons. Cardiologia **4:** 1, 1940.

(123) GROEDEL, F. M. AND P. R. BORCHARDT. Extrasystoles in man produced by direct mechanical irritation of the left or right ventricle. Exper. Med. and Surg. **4:** 145, 1946.

(124) GROEDEL, F. M., B. KISCH AND P. REICHERT. Changes in the standard electrocardiogram and the chest leads during the first stages of life. Cardiologia **6:** 1, 1942.

(125) GROEDEL, F. M. AND E. KOCH. Tierversuche zur Frage des Partial-Elektrokardiogramms der rechten und der linken Herzkammer. Ztschr. f. Kreislauf. **25:** 794, 1933.

(126) HAAGER, B. AND A. WEBER. Klinische und experimentelle Untersuchung über das Elektrokardiogramm; über monophasiche Ableitung vom Tierherzen. Ztschr. f. klin. Med. **131:** 136, 1936.

(127) HARRIS, A. S. Spread of excitation in turtle heart as measured by remote and exploring electrodes and two contiguous electrodes. Am. J. Physiol. **129:** 376,1940.

(128) HARRIS, A. S. The spread of excitation in turtle, dog, cat and monkey ventricles. Am. J. Physiol. **134:** 319, 1941.

(129) HECHT, H. H. The influence of the indifferent electrode upon the precordial electrocardiogram; I. The normal electrocardiogram. Am. Heart J. **24:** 529, 1942.

(130) HECHT, H. H. Potential variations of the right auricular and ventricular cavities in man. Am. Heart J. **32:** 39, 1946.

(131) HELM, J. D., JR., G. H. HELM AND C. C. WOLFERTH. The distribution of potential of ventricular origin below the diaphragm and in the esophagus. Am. Heart J. **27:** 755, 1944.

(132) HERVE L. AND F. HUIDOBRO. Posición del corazón y forma de extrasístoles. Anal. Acad. Biol. Univ. Chile **3:** 43, 1939.

(133) HESS, W. Modellversuche über den Verlauf der Potentiallinien des Herzens im transversalen Brustkorbquerschnitt. Ztschr. f. Kreislauff. **27:** 433, 1935.

(134) HILL, A. Analysis of the normal QRS deflection. Lancet **2:** 1110, 1938.

(136) HILL, I. G. W., F. D. JOHNSTON AND F. N. WILSON. The form of the electrocardiogram in experimental myocardial infarction. Am. Heart J. **16:** 309, 1938.

(137) HILL, R. F. The construction of the cardiac vector. Am. Heart J. **32:** 72, 1946.

(138) HOFF, E. C., T. C. KRAMER, D. DUBOIS AND B. M. PATTEN. The development of the electrocardiogram of the embryonic heart. Am. Heart J. **17:** 470, 1939.

(139) HOFF, H. E. AND L. H. NAHUM. The supernormal period in the mammalian ventricle. Am. J. Physiol. **124:** 591, 1938.

(140) HOFF, H. E. AND L. H. NAHUM. Influence of local applications of potassium chloride on action current of the mammalian heart. Proc. Soc. Exper. Biol. and Med. **45:** 263, 1940.

(141) Hoff, H. E. and L. H. Nahum. The factors determining the direction of the T wave; the effect of heat and cold upon the dextro- and levo-cardiogram. Am. J. Physiol. **131:** 700, 1941.

(142) Hoff, H. E. and L. H. Nahum. A comparison of the configuration in the electrocardiogram of endocardial and epicardial extrasystoles. Am. J. Physiol. **140:** 148, 1943.

(143) Hoff, H. E. and L. H. Nahum. The electrocardiographic localization of myocardial infarcts by injury currents and ventricular extrasystoles. Am. J. Physiol. **143:** 723, 1945.

(144) Hoff, H. E., L. H. Nahum and W. Kaufman. The nature of leads I and III of the electrocardiogram. Am. J. Physiol. **134:** 390, 1941.

(145) Hoff, H. E., L. H. Nahum and W. Kaufman. The nature of QI and QIII. Am. J. Physiol. **135:** 752, 1942.

(146) Hoff, H. E., L. H. Nahum and W. Kaufman. Distribution in leads I, II, and III of potentials applied to the surface of the heart. Am. J. Physiol. **138:** 644, 1943.

(147) Hoff, H. E., L. H. Nahum and B. Kisch. Influence of right and left ventricles on the electrocardiogram. Am. J. Physiol. **131:** 687, 1941.

(148) Hogg, M. B., C. M. Goss and K. S. Cole. Potentials in embryo rat heart muscle cultures. Proc. Soc. Exper. Biol. and Med. **32:** 304, 1935.

(149) Hollmann, H. E. and W. Hollmann. Das Einthoven'sche Dreiecksschema als Grundlage neuer elektrokardiographischer Registriermethoden. Ztschr. f. klin. Med. **134:** 732, 1938.

(150) Hollmann, H. E. and W. Hollmann. Das Einthovensche Dreiecksschema im Vergleich zu anderen Ableitungsschemen. Arch. f. Kreislauff. **3:** 191, 1938.

(151) Hollmann, H. E. and E. Guckes. Das Triogram und seine klinische Bedeutung. Arch. f. Kreislauff. **4:** 69, 1939.

(152) Hollmann, W. and H. E. Hollmann. Neue Elektrokardiographische Untersuchungsmethoden. Ztschr. f. Kreislauff. **29:** 546, 1937.

(153) Holzmann, M. Neuere praktische Ergebnisse der Elektrokardiographie. Schweiz. med. Wchnschr. **69:** 289, 1939.

(154) Howard, F. H. A method for the construction of the vectocardiogram from the Einthoven electrocardiogram. Am. Heart J. **31:** 191, 1946.

(155) Jervell, A. Über die theoretische Grundlage der Elektrokardiographie und ihre praktische Bedeutung. Acta Med. Scandinav. **78:** 41, 1936.

(156) Jochim, K., L. N. Katz and W. Mayne. The monophasic electrogram obtained from the mammalian heart. Am. J. Physiol. **111:** 177, 1935.

(157) Johnston, F. D., I. G. W. Hill and F. N. Wilson. The form of the electrocardiogram in experimental myocardial infarction. Am. Heart J. **10:** 889, 1935.

(158) Johnston, F. D. and F. N. Wilson. A vacuum tube integrator for measuring the areas of waves of the electrocardiogram. Proc. Centr. Soc. Clin. Res. **16:** 55, 1943.

(159) Katz, L. N. The asynchronism of right and left ventricular contractions and the independent variations in their duration. Am. J. Physiol. **72:** 655, 1925.

(160) Katz, L. N. Effect on electrocardiogram of opening the thorax and inserting optical manometers into the aorta and pulmonary artery. Proc. Soc. Exper. Biol. and Med. **24:** 652, 1927.

(161) Katz, L. N. The significance of the T wave in the electrogram and electrocardiogram. Physiol. Rev. **8:** 447, 1928.

(162) Katz, L. N. Recent advances in the interpretation of the electrocardiogram. J.A.M.A. **97:** 1364, 1931.

(163) Katz, L. N. Concerning a new concept of the genesis of the electrocardiogram. Am. Heart J. **13:** 17, 1937.

(164) Katz, L. N. Electrocardiography. Lea & Febiger, Philadelphia, 2nd ed., 1946.

(165) KATZ, L. N. AND W. ACKERMAN. The effect of the heart's position on the electrocardiographic appearance of ventricular extrasystoles. J. Clin. Investigation **11:** 1221, 1932.

(166) KATZ, L. N., A. M. GOLDMAN, R. LANGENDORF, L. G. KAPLAN AND S. T. KILLIAN. The diagnostic value of the electrocardiogram based on an analysis of 149 autopsy cases. Am. Heart J. **24:** 627, 1942.

(167) KATZ, L. N., I. GUTMAN AND F. H. OCKO. Alterations of the electrical field produced by changes in the contacts of the heart with the body. Am. J. Physiol. **116:** 302, 1936.

(168) KATZ, L. N., K. JOCHIM AND A. GOLDMAN. The effect of an injured area on the electrical field of the heart based on experiments with models. Am. J. Physiol. **137:** 779, 1942.

(169) KATZ, L. N. AND H. KOREY. The manner in which the electric currents generated by the heart are conducted away. Am. J. Physiol. **111:** 83, 1935.

(170) KATZ, L. N. AND R. LANGDENDORF. The value of chest leads CF_2, CF_4 and CF_5 in the evaluation of heart strain. Arch. Inst. Cardiologica México **15:** 43, 1945.

(171) KATZ, L. N., H. LANDT AND A. BOHNING. The delay in the onset of ejection of the left ventricle in bundle branch block. Am. Heart J. **10:** 681, 1935.

(172) KATZ, L. N. AND M. ROBINOW. The appearance of the electrocardiogram in relation to the position of the heart within the chest. Am. J. Med. Sci. **192:** 556, 1936.

(173) KATZ, L. N., E. SIGMAN, I. GUTMAN AND F. H. OCKO. The effect of good electrical conductors introduced near the heart on the electrocardiogram. Am. J. Physiol. **116:** 343, 1936.

(174) KATZ, L. N. AND A. W. WALLACE. The rôle of cardiac ischemia in producing R-T deviations in the electrocardiogram. Am. J. Med. Sci. **181:** 836, 1931.

(175) KATZ, L. N. AND S. F. WEINMAN. The relation of the T wave to the asynchronism between the ends of right and left ventricular ejection. Am. J. Physiol. **81:** 360, 1927.

(176) KAUFMAN, W. AND F. D. JOHNSTON. The electrical conductivity of the tissues near the heart and its bearing on the distribution of the cardiac action currents. Am. Heart J. **26:** 42, 1943.

(177) KELLOGG, O. P. Foundations of potential theory. F. Ungar Publ. Co., New York, 1943.

(178) KEITH, A. The sino-auricular node; a historical note. Brit. Heart J. **4:** 77, 1942.

(179) KIENLE, F. Untersuchungen über ein neues Verfahren zur Ableitung des Elektrocardiogramms von der Brustwand unter Anwendung einer differenten und einer indifferenten Elektrode. Arch. f. Kreislauff. **2:** 224, 1938.

(181) KISCH, B. The influence of the surface of heart muscle on the electrocardiogram. Cardiologia **4:** 304, 1940.

(182) KISCH, B. The changes of the chest leads of the electrocardiogram due to damage of the heart's surface. Cardiologia **4:** 318, 1940.

(183) KISCH, B., L. H. NAHUM AND H. E. HOFF. The predominance of surface over deep cardiac injury in producing changes in the electrocardiogram. Am. Heart J. **20:** 174, 1940.

(184) KISSIN, M., W. ACKERMAN AND L. N. KATZ. The effect of the heart's position on the electrocardiographic appearance of bundle branch block in man. Am. J. Med. Sci. **186:** 721, 1933.

(185) KOCH-MOMM, E. Experimenteller Beitrag zur Theorie des Elektrokardiogramms. Ztschr. f. Kreislauff. **24:** 501, 1932.

(186) KOCH-MOMM, E. Die Ungültigkeit des Einthoven'schen Dreieckschemas für das Elektrocardiogramm. Ztschr. f. Kreislauff. **25:** 513, 1933.

(187) KOCH-MOMM, E. Modellversuche über den Einfluss des elektrischen Feldes auf die Ableitung von Potentialdifferenzen. Ztschr. f. Biol. **93:** 241, 1933.

(188) KOREY, H. AND L. N. KATZ. The electrocardiographic changes produced by injuries of various parts of the ventricles. Am. J. Med. Sci. **188:** 387, 1934.

(189) KOSSMANN, C. E. AND F. D. JOHNSTON. The precordial electrocardiogram. I. The potential variations of the precordium and the extremities in normal subjects. Am. Heart J. **10:** 925, 1935.

(190) KOUNTZ, W. B. AND M. PRINZMETAL. Revival of the human heart. J. Clin. Investigation **13:** 711, 1934.

(191) KOUNTZ, W. B. AND M. PRINZMETAL. Influence of inversion of the dog on the electrocardiogram. Proc. Soc. Exper. Biol. and Med. **31:** 612, 1934.

(192) KOUNTZ, W. B., M. PRINZMETAL, E. F. PEARSON AND K. F. KOENING. The effect of position of the heart on the electrocardiogram. I. The electrocardiogram in revived perfused human hearts in normal position. Am. Heart J. **10:** 605, 1935.

(193) KOUNTZ, W. B., M. PRINZMETAL AND J. R. SMITH. The effect of position of the heart on the electrocardiogram. II. Observations upon the electrocardiogram obtained from a dog's heart placed in the human pericardial cavity. Am. Heart J. **10:** 614, 1935.

(194) KOUNTZ, W. B., M. PRINZMETAL AND J. R. SMITH. The effect of position of the heart on the electrocardiogram. III. Observations upon the electrocardiogram in the monkey. Am. Heart J. **10:** 623, 1935.

(195) KRASNO, M. R., J. A. E. EYSTER AND J. P. HETTWER. Action potentials from the heart of the early chick embryo. Am. J. Physiol. **119:** 357, 1937.

(196) KRASNO, M. R., J. A. E. EYSTER AND C. A. MAASKE. The nature of the T wave potentials in the tortoise heart. Am. J. Physiol. **114:** 119, 1935.

(197) LEPESCHKIN, E. Über das normale Brustwand-Elektrokardiogramm im Kindesalter. Arch. f. Kreislauff. **3:** 321, 1938.

(198) LEWIS, T. The mechanism and graphic registration of the heart beat. Shaw & Sons, Ltd., London, 1925, 3rd ed.

(199) LEWIS, T. AND M. A. ROTHSCHILD. The excitatory process in the dog's heart. II. Phil. Tr. London, Series B. **206:** 181, 1915.

(200) LINDNER, E. AND L. N. KATZ. The relative conductivity of the tissues in contact with the heart. Observations on animals with closed chests. Am. J. Physiol. **125:** 625, 1939.

(201) LLOYD, W. The form and function of the auriculo-ventricular bundle in the rabbit. Am. J. Anat. **45:** 379, 1930.

(202) LOMBARDINI, R. V. Une autre théorie sur l'onde T. Arch. Mal. du Coeur **26:** 548, 1933.

(203) LUNDY, C. J. AND C. M. BACON. Premature left ventricular beats from electrical stimulation of exposed human heart. Arch. Int. Med. **52:** 30, 1933.

(204) LUNDY, C. J., I. TREIGER AND R. DAVISON. Ventricular extrasystoles induced by electrical stimulation of the exposed human heart rotated 30° counterclockwise on its vertical axis. Am. Heart J. **17:** 85, 1939.

(205) MACLEOD, A. G. The electrogram of cardiac muscle. I. An analysis which explains the regression of T deflection. Am. Heart J. **15:** 165, 1938.

(206) MACLEOD, A. G. The electrogram of cardiac muscle. II. The lengths of the stages of activity. Am. Heart J. **15:** 402, 1938.

(207) MACLEOD, A. G., F. N. WILSON AND P. S. BARKER. The form of the electrocardiogram. I. Intrinsicoid electrocardiographic deflections in animals and man. Proc. Soc. Exper. Biol. and Med. **27:** 586, 1930.

(208) MAHAIM, I. Les maladies organiques du faisceau de His-Tawara. Masson & Cie., Paris, 1931.

(209) MAHAIM, I. Le syndrome de Wolff-Parkinson-White et sa pathogenie. Helvetica Med. Acta. **8:** 483, 1941.

(210. MAHAIM, I. AND M. R. WINSTON. Researches on the comparative anatomy and the experimental pathology of the superior connection of the bundle of His-Tawara. Cardiologia **5:** 189, 1941.

(211) MAHAIM, I., M. R. WINSTON AND H. ROESLER. Bilateral missed block, report of a case in which there was left bundle branch block during life with autopsy. Am. Heart J. **25:** 251, 1943.

(212) MANN, H. A method of analyzing the electrocardiogram. Arch. Int. Med. **25:** 283, 1920.

(213) MANN, H. The monocardiograph. Am. Heart J. **15:** 681, 1938.

(214) MARVIN, H. M. AND A. W. OUGHTERSON. The form of premature beats resulting from direct stimulation of the human ventricle. Am. Heart J. **7:** 471, 1932.

(215) MEEK, W. J., J. A. E. EYSTER AND H. GOLDBERG. Certain characteristics of injury potential in cardiac muscle. Am. J. Physiol. **126:** 583, 1939.

(216) MEHRING, C. E. AND K. E. ROTHSCHUH. Weiterer Untersuchungen über das elektrische Aktionsphenomena des Herzens. Ztschr. f. Biol. **100:** 68, 1940.

(217) MILLER, J. R. AND R. F. DENT. A new hypothesis of the production of the T wave in the electrocardiogram based on electro-kinetic phenomena. J. Clin. Investigation **19:** 783, 1940.

(218) MOLZ, B. Modellversuche zur kenntnis des elektrischen Feldes in der Elektrokardiographie. Zur Frage der Nullpotential-Elektrode und des Einthovenschen Dreieckschemas. Pflüger's Arch. **237:** 251, 1936.

(219) MOLZ, B. Eine Methode der unipolaren Ableitung des Elektrokardiogramms. Ztschr. f. Kreislauff. **29:** 361, 1937.

(220) MOLZ, B. Über die unipolare Ableitung des Elektrokardiogramms. Pflüger's Arch. **242:** 416, 1939.

(221) MÖNCKEBERG, J. G. Erkrankungen des Myocards und des spezifischen Muskelsystems. Handb. der sp. Path. Anat. und Histologies. J. Springer, Berlin, 1924.

(222) MYERS, G. B. AND B. G. OREN. The use of the augmented unipolar left leg lead in the differentiation of the normal from abnormal Q wave in standard lead III. Am. Heart J. **29:** 708, 1945.

(223) NACHMANSOHN, D. On the mechanism of transmission of nerve impulses. Collect. Net. **17:** August 1942.

(224) NACHMANSOHN, D., R. T. COX, C. W. COATES AND A. L. MACHADO. Phosphocreatine as energy source of the action potential. Proc. Soc. Exper. Biol. and Med. **52:** 97, 1943.

(225) NAHUM, L. H., W. F. HAMILTON AND H. E. HOFF. The injury current in the electrocardiogram. Am. J. Physiol. **139:** 202, 1943.

(226) NAHUM, L. H. AND H. E. HOFF. The recovery of electrical excitability in the mammalian ventricle. Am. J. Physiol. **126:** 589, 1939.

(227) NAHUM, L. H. AND H. E. HOFF. The interpretation of the U wave of the electrocardiogram. Am. Heart J. **17:** 585, 1939.

(228) NAHUM, L. H. AND H. E. HOFF. The localization of ventricular extrasystoles. Yale J. Biol. and Med. **17:** 539, 1945.

(229) NAHUM, L. H., H. E. HOFF AND W. KAUFMAN. Formation of the R complex of the electrocardiogram. Am. J. Physiol. **133:** 396, 1941.

(230) NAHUM, L. H., H. E. HOFF AND W. KAUFMAN. Configuration of anterior and posterior septal extrasystoles in the standard leads of the electrocardiogram. Am. J. Physiol. **134:** 398, 1941.

(231) NAHUM, L. H., H. E. HOFF AND W. KAUFMAN. Influence of temperature on the electrogram and monophasic action potential of the mammalian heart. Proc. Soc. Exper. Biol. and Med. **46:** 395, 1941.

(232) NAHUM, L. H., H. E. HOFF AND W. KAUFMAN. Formation of the R wave of the electrocardiogram. Am. J. Physiol. **134:** 384, 1941.

(233) NAHUM, L. H., H. E. HOFF AND W. KAUFMAN. The nature of the S complex of the electrocardiogram. Am. J. Physiol. **136:** 726, 1942.

(234) NAHUM, L. H., H. E. HOFF AND B. KISCH. The significance of displacement of the RS-T segment. Am. J. Physiol. **131:** 693, 1941.

(235) NONIDEZ, J. F. The structure and innervation of the conductive system of the heart of the dog and rhesus monkey, as seen with a silver impregnation technique. Am. Heart J. **26:** 577, 1943.

(236) NYBOER, J. Further observations on the esophageal electrocardiogram. J. Clin. Investigation **21:** 649, 1942.

(237) NYLIN, G. AND C. CRAFOORD. Simultaneous electrograms from left and right ventricles of the human heart. Cardiologia **6:** 136, 1942.

(238) NYLIN, G. AND K. E. GREWIN. The chest leads and their value in association with the leads from the extremities, and several other chest leads. Cardiologia **6:** 169, 1942.

(239) NYLIN, G. AND T. SÄLLSTRÖM. Three synchronized leads between fixed points on the heart projection on the chest wall. Acta med. Scand. **96:** 1, 1938.

(240) ÖHNELL, R. F. Pre-excitation, a cardiac abnormality. Acta med. Scand. Suppl. **152:** 1, 1944.

(241) PANNIER, R. Le tissue Purkinien des oreillettes du chat. Arch. Biol. **50:** 271, 1939.

(242) PANNIER, R. AND N. GOORMAGHTIGH. Sur l'existence de faisceaux Purkiniens dans l'oreillette droite. C. R. Soc. Biol. **127:** 1114, 1938.

(243) PAPP, C. U, the 6th wave of the electrocardiogram. Brit. Heart J. **2:** 9, 1940.

(244) PATTEN, B. M. Microcinematographic and electrocardiographic studies of the first heart beats and the beginning of the circulation in living embryos. Proc. Inst. Med. Chicago, **12:** 366, 1939.

(245) PATTEN, B. M. AND T. C. KRAMER. The initiation of contraction in the embryonic chick heart. Am. J. Anat. **53:** 349, 1933.

(246) PARDEE, H. E. B. AND M. GOLDENBERG. Electrocardiographic features of myocardial infarction as affected by involvement of the septum and by complete and incomplete transmural involvement. Am. Heart J. **30:** 367, 1945.

(247) PEARSON, H. E. S. AND L. M. GERLIS. A quantitative electrocardiographic method. Brit. Heart J. **7:** 173, 1945.

(248) POL, VAN DER, B. AND J. VAN DER MARK. Le battement du coeur considéré comme oscillation de relaxation et un modèle életrique due coeur. L'onde electrique **7:** 365, 1928 (also Arch. Neer. Physiol. **14:** 418, 1929).

(248a) PRINZMETAL, M., W. B. KOUNTZ AND D. P. BARR. The contour of the chest as a factor in the explanation of the difference between the dog and human electrocardiogram. J. Clin. Investigation **13:** 695, 1934.

(249) PRINZMETAL, M., W. B. KOUNTZ AND L. GOTTLIEB. Influence of point at which ventricle contacts thoracic wall on form of the electrocardiogram. Proc. Soc. Exper. Biol. and Med. **32:** 1411, 1935.

(250) PRINZMETAL, M., B. S. OPPENHEIMER AND S. DACK. Localization of ventricular extrasystoles. J.A.M.A. **108:** 620, 1937.

(251) PRUITT, R. D., A. R. BARNES AND H. E. ESSEX. Electrocardiographic changes associated with lesions in the deeper layers of the myocardium. Am. J. Med. Sci. **210:** 100, 1945.

(252) PUDDU, V. AND C. CAMMARELLA. Un cas d'ectopia cordis avec une étude electrocardiographique de la progression de l'onde d'excitation. Arch. Mal. du Coeur **31:** 861, 1938.

(253) RAPPORT, D. AND G. B. RAY. Changes of electrical conductivity in the beating tortoise ventricle. Am. J. Physiol. **80:** 126, 1927.

(254) RAWLINSON, H. E. The later development of the sinus venosus and the relation of the sino-atrial node to it, in the calf heart. Anat. Rec. **49:** 89, 1931.

(255) RIJLANT, P. Contribution à l'étude de l'automatisme et de la conduction dans le coeur. II. Conduction intra-auriculaire chez le mammifère. Arch. Intern. Physiol. **27:** 225, 1926.

(256) RIJLANT, P. La conduction dans l'oreillette droite du coeur du mammifère. C. R. Soc. Biol. **103:** 909, 1930.

(257) RIJLANT, P. La conduction dans le coeur du mammifère. Ann. Physiol. et Physico-Chim. Biol. **7:** 229, 1931.

(258) RIJLANT, P. La conduction dans le coeur du mammifère. Arch. Internat d. Physiol. **33:** 325, 1931.

(259) Rijlant, P. Mécanisme de l'envahissement de l'oreillete droite du coeur du mammifère par la contraction. C.R. Soc. Biol. **121:** 1361, 1936.

(260) Rijlant, P. Introduction à l'étude de la distribution spatiale des variations de potentiel produites par le coeur chez l'homme. C.R. Soc. Biol. **121:** 1358, 1936.

(261) Robb, J. S. Evidence for characteristic modifications of the electrocardiogram produced by lesions of ventricular muscle bands. Proc. Soc. Exper. Biol. and Med. **31:** 311, 1933.

(262) Robb, J. S. The structure of the mammalian auricle. Med. Women's J. **41:** 65, 1934.

(263) Robb, J. S. The distribution of the Purkinje material in the septum of the beef heart. Am. J. Physiol. **126:** 608, 1939.

(264) Robb, J. S. A study of Q-T interval in various species. Fed. Proc. **5:** 87, 1946.

(265) Robb, J. S., M. S. Dooley and R. C. Robb. Displacement of the RS-T segment by potassium chloride. J. Mt. Sinai Hosp. **8:** 946, 1942.

(266) Robb, J. S., M. Easby and J. G. F. Hiss. Experimental interference with conduction in the heart. Am. J. Med. Sci. **188:** 835, 1934.

(267) Robb, J. S., W. Greene and R. C. Robb. The peripheral distribution of the Purkinje fibres. J. Tech. Methods **17:** 91, 1937.

(268) Robb, J. S. and J. G. F. Hiss. Experimental interference with conduction in the ventricle. Am. J. Physiol. **109:** 89, 1934.

(269) Robb, J. S., J. G. F. Hiss and R. C. Robb. Localization of cardiac infarcts according to component ventricular muscles. Am. Heart J. **10:** 287, 1935.

(270) Robb, J. S. and C. T. Kaylor. The A-V conduction system in the heart of the guinea pig. Proc. Soc. Exper. Biol. and Med. **59:** 92, 1945.

(271) Robb, J. S. and R. C. Robb. The excitatory process in the mammalian ventricle. Am. J. Physiol. **115:** 43, 1936.

(272) Robb, J. S. and R. C. Robb. The conducting system. Am. J. Physiol. **119:** 390, 1937.

(273) Robb, J. S. and R. C. Robb. Converse effects of stimulating opposite ends of a cardiac muscle band. Proc. Soc. Exper. Biol. and Med. **37:** 187, 1937.

(274) Robb, J. S. and R. C. Robb. The S wave of the electrocardiogram. Am J. Physiol. **126:** 608, 1939.

(275) Robb, J. S. and R. C. Robb. Hypertension electrocardiograms experimentally produced and anatomically explained. I. Cor pulmonale. Am. J. Med. Sci. **203:** 625, 1942.

(276) Robb, J. S. and R. C. Robb. Hypertension electrocardiograms experimentally produced and anatomically explained. II. Left ventricular strain. Am. J. Med. Sci. **203:** 634, 1942.

(277) Robb, J. S., R. C. Robb and J. G. F. Hiss. Localization of premature beats in the mammalian ventricle. Proc. Soc. Exper. Biol. and Med. **32:** 1510, 1935.

(278) Robb, R. C. and J. S. Robb. Experimental bundle branch block after ablation of one or both ventricles. Am. J. Med. Sci. **204:** 313, 1942.

(279) Robb, J. S. and R. C. Robb. The normal heart. Anatomy and physiology of the structural unit. Am. Heart J. **23:** 455, 1942.

(280) Roberts, G. H., J. C. Hamilton and D. I. Abramson. Experimental bundle branch block in the monkey. J. Clin. Investigation **14:** 867, 1935.

(281) Robinow, M. A proved case of dextroposition of the heart showing left axis deviation in the electrocardiogram. Am. Heart J. **13:** 104, 1937.

(282) Robinow, M., L. N. Katz and A. Bohning. The appearance of the T wave in lead IV in normal children and in children with rheumatic heart disease. Am. Heart J. **12:** 88, 1936.

(283) Rochet, J. and M. M. Vastesaeger. Le vectocardiogramme de l'homme normal, Travaux Labor. Inst. Solvay Physiol. **29:** 17, 1944.

(284) Rojas, P. and J. Debussy-Schleisinger. El tejido conectivo de los distintos segmentos del sistema de conducción auriculo-ventricular. Rev. Soc. Argent. Biol. **12:** 313, 1936.

(285) Rosenbaum, F. F., F. N. Wilson and F. D. Johnston. The precordial electrocardiogram in high lateral myocardial infarction. Proc. Cent. Soc. Clin. Res. **18:** 35, 1945.

(286) Rosenblueth, A., W. Daŭghaday and D. D. Bond. The action of electrial stimuli on the turtle's ventricle. Am. J. Physiol. **138:** 50, 1942.

(287) Rosenblueth, A., W. Daughaday and D. D. Bond. The electrogram of the ventricle of the turtle's heart. Am. J. Physiol. **139:** 464, 1943.

(288) Rosenblueth, A. and E. C. del Pozo. The changes of impedance of the turtle's ventricular muscle during contraction. Am. J. Physiol. **139:** 514, 1943.

(289) Rosenblueth, A. and J. Garcia-Ramos. La interpretación del electrograma del ventrículo cardiaco. Arch. Inst. Cardiologica México **14:** 232, 1945.

(290) Rosenblueth, A. and J. Garcia-Ramos. La acción de la corriente directa sobre los diversos componentes del electrograma del ventrículo cardíaco. Arch. Inst. Cardiologica México **15:** 1, 1945.

(291) Rosenblueth, A. and J. García-Ramos. La acción del calor y el frío sobre los diversos componentes del electrograma del ventrículo cardíaco. Arch. Inst. Cardiologica México **15:** 101, 1945.

(292) Rosenblueth, A. and J. Garcia-Ramos. Los diversos componentes del electrograma monofásico del ventrículo cardíaco. Arch. Inst. Cardiologica México **16:** 45, 1946.

(293) Rothberger, C. J. Beiträge zur normalen and pathologischen Physiologie der spezifischen Herzmuskulatur. Cardiologia **1:** 234, 1937.

(294) Rothberger, C. J. Normale and pathologische Physiologie der Rhythmik und Koordination des Herzens. Ergbn. d. Physiol. **32:** 472, 1931.

(295) Rothschuh, K. E. Über die Entstehung von Verletzungsströmen und monophasischer Derformierung der Herzaktions-Ströme durch Körpereigene Substanzen. Ztschr. f. exper. Med. **106:** 543, 1939.

(296) Rothschuh, K. E. Über die Entstehung von Q- and S-Zacken im Elektrokardiogram. Pflüger's Arch. **246:** 820, 1943.

(297) Routier, D. Le vectogramme. Arch. Mal. du Coeur **31:** 697, 1938.

(298) Sanabria, T. Recherches sur la differenciation du tissu nodal et connecteur du coeur des Mammíferes. Arch. Biol. **47:** 1, 1935.

(299) Schaefer, H. Elektrophysiologie, II. Band: Spezielle Elektrophysiologie; F. Deuticke, 1942, Lithoprinted Edward Bros. Inc., Ann Arbor, 1944, pp. 1 to 130 and pp. 331 to 348.

(300) Schaefer, H. Theorie des Potentialabgriffs beim Elektrokardiogramm auf der Grundlage der Membran-Theorie. Pflüger's Arch. **245:** 72, 1941.

(301) Schaefer, H., A. Pena and P. Schölmerich. Der monophasiche Aktionsstrom der Spitze und Basis des Warmblüterherzens und die Theorie der T Welle des Ekg. Pflüger's Arch. **246:** 728, 1943.

(302) Schellong, F. Vektordiagraphie des Herzens als klinische Methode. Klin. Wschr. **17:** 453, 1938.

(303) Schellong, F., S. Heller and E. Schwinge. Das Vektordiagramm; eine Untersuchungs-Methode des Herzens. Ztschr. f. Kreislauff. **29:** 497, 1937.

(304) Schellong, F. and E. Schwinge. Das Vektordiagramm; eine Untersuchungsmethode des Herzens; über die Bedeutung von Knotungen und Aufsplitterung in QRS des Ekg. Ztschr. f. Kreislauff. **29:** 596, 1937.

(305) Schellong, F., E. Schwinge and H. Hermann. Die praktisch-klinische Methode der Vektordiagraphie und das normale Vektordiagramm. Arch. f. Kreislauff. **2:** 1, 1937.

(306) Schocken, K. The validity of Einthoven's triangle rule. Cardiologia **3:** 197, 1939.

(307) Schütz, E. Monophasische Aktionsströme vom in situ durchbluteten Säugetierherzen. Klin. Wchnschr. **31:** 1454, 1931.

(308) Schütz, E. Einphasische Aktionsströme vom in situ durchbluteten Säugetierherzen. Ztschr. f. Biol. **92:** 441, 1932.

(309) Schütz, E. Elektrophysiologie des Herzens bei einphasischer Ableitung. Ergebn. d. Physiol. **38:** 493, 1936.

(310) Schütz, E., K. E. Rothschuh and C. E. Mehring. Ein direkter Beweis für die Gültigkeit der Differenzkonstruktion des Elektrogramms. Klin. Wchnschr. **19:** 9, 1940.

(311) Segers, M. Le parallélisme entre l'accommodation de l'excitabilité et du rythme du coeur. Acta Biol. Belg. **1:** 255, 1941.

(312) Segers, M. L'identité de l'onde U et de la négativité tardive du coeur. Acta Biol. Belg. **3:** 72, 1943.

(313) Shaner, R. F. The development of the atrio-ventricular node, bundle of His, and sino-atrial node in the calf, with a description of a third embryonic node-like structure. Anat. Rec. **44:** 85, 1929.

(314) Sodi Pallares, D. Nuevas bases de electrocardiografía. Talleres Gráficos de la Nación, Mexico, D. F., 1945, Ediciones del Instituto Nat. de Cardiología.

(315) Sodi Pallares, D., A. Cuellar and E. Cabrera. Sistema de seis ejes con aplicación al vector AvT en las hipertrofias ventriculares. Arch. Inst. Cardiologica México **14:** 142, 1945.

(316) Stratton, J. A. Electromagnetic theory. McGraw-Hill Book Co., N.Y.C., 1941.

(317) Storti, E. Zur Frage der Nullpotential Elektrode in der Elektrokardiographie. Ztschr. f. Kreislauff. **27:** 830, 1935.

(318) Sugarman, H., L. N. Katz, A. Sanders and K. Jochim. Observations on the genesis of the electrical currents established by injury to the heart. Am. J. Physiol. **130:** 130, 1940.

(319) Sulzer, R. and P. W. Duchosal. L'électrocardiographie à deux dimensions ou planélectrocardiographie. Helvet. Med. Acta. **4:** 740, 1937.

(320) Sulzer, R. and P. W. Duchosal. Application de la planographie à l'étude de la distribution des potentiels du coeur à la surface du corps. Arch. Mal. d. Coeur **31:** 686, 1938.

(321) Sulzer, R. and P. W. Duchosal. Die Grundform des menschlichen Vektorkardiogramms. Schweiz. Med. Wchnschr. **71:** 259, 1941.

(322) Sulzer, R. and P. W. Duchosal. Principle of cardiovectography. Cardiologia **6:** 236, 1942.

(323) Sulzer, R. and P. W. Duchosal. Le cardiovectogramme normal. Helv. Physiol. Acta. **4:** 285, 1946.

(324) Szepesenwol, J. and J. B. Odoriz. Potenciales de acción de esbozo cardíaco de embriones de pollo y de rata cultivádos in vitro. Rev. Soc. Argent. Biol. **19:** 279, 1943.

(325) Taussig, H. B. Boundaries of sino-auricular node and atrio-ventricular node in human heart. Johns Hopkins Hosp. Bull. **48:** 162, 1931.

(326) Ter Borg, H. (Purkinje tissue in the atria of domestic animals (esp. the horse)). Acta. Neer. Morph. **1:** 64, 1937.

(327) Tigerstedt, R. Die Physiologie des Kreislaufes. W. de Gruyter, Berlin, 1921. (4 vol.)

(328) Vastesaeger, M. M. and J. Rochet. Les propriétés du vectocardiogramme spatial de l'homme normal, ses variations physiologiques. Trav. Lab. Inst. Solvay Physiol. **29:** 55, 1944.

(329) Vastesaeger, M. M. and J. Rochet. La stereovectocardiographie et la stereovectocardioscopie, méthodes cliniques d'étude de la repartition spatiale des potentiels cardiaques. Trav. Lab. Inst. Solvay Physiol. **29:** 40, 1944.

(330) Wahlin, B. Das Reizleitungssystem und die Nerven des Säugetierherzens. Inaug. Dissert. Stockholm, 1935, also Sv. Lak. Sallsk. Hdl. **62:** 1, 1936.

(331) Waller, A. On the electromotive changes connected with the beat of the mammalian heart, and of the human heart in particular. Phil. Trans. Royal Soc. London, Series B. **180:** 169, 1889.

(332) Walls, E. W. Specialised conducting tissue in heart of golden hamster. J. Anat. **76:** 359, 1942.

(333) WALLS, E. W. Specialised conducting tissue in heart of common hedgehog. J. Anat. **77:** 294, 1943.

(334) WALLS, E. W. Dissection of atrio-ventricular node and bundle in human heart. J. Anat. **79:** 45, 1945.

(335) WEBER, A. Klinische und experimentelle Studien über das Elektrokardiogramm; Experimentelle Untersuchungen zur Deutung des Elektrokardiogramms. Ztschr. klin. Med. **132:** 153, 1937.

(336) WEDD, A. M. AND W. D. STROUD. The spread of the excitation wave related to the standard electrocardiogram in the dog's heart. Heart **9:** 15, 1921.

(337) WEINSTEIN, J. AND D. I. ABRAMSON. Localization of the site of experimental premature contractions and bundle branch lesions by means of multiplane chest leads. Ann. Int. Med. **9:** 1187, 1936.

(338) WENCKEBACH, K. F. AND H. WINTERBERG. Die unregelmässige Herztätigkeit. W. Engelmann, Leipzig, 1937.

(339) WIGGERS, C. J. The independence of electrical and mechanical reactions in the mammalian heart. Am. Heart J. **1:** 173, 1925.

(340) WIGGERS, C. J. Monophasic and deformed ventricular complexes resulting from surface applications of potassium salts. Am. Heart J. **5:** 346, 1930.

(341) WIGGERS, C. J. AND M. G. BANUS. A. On the independence of electrical and mechanical activity in the mammalian ventricle. B. On the effects of pH changes on conduction in the heart. Am. J. Physiol. **76:** 215, 1926.

(342) WIGGERS, H. C. Pure monophasic action potentials and their employment in studies of ventricular surface negativity. Proc. Soc. Exper. Biol. and Med. **34:** 337, 1936.

(343) WIGGERS, H. C. The sequence of ventricular surface excitation determined by registration of monophasic action potentials. Am. J. Physiol. **118:** 333, 1937.

(344) WIGGERS, H. C. The time relationship of electrical and mechanical processes in the ventricle of the dog. Am. J. Physiol. **123:** 215, 1938.

(345) WIGGERS, H. C. AND C. J. WIGGERS. The interpretation of monophasic action potentials from the mammalian ventricle indicated by changes following coronary occlusion. Am. J. Physiol. **113:** 683, 1935.

(346) WILDE, W. S., C. E. DRAWE, AND R. ASHMAN. The sequence of excitation of the surface of the turtle ventricle. Am. J. Physiol. **129:** 497, 1940.

(347) WILSON, F. N. The distribution of potential differences produced by the heart beat within the body and at its surface. Am. Heart J. **5:** 599, 1930.

(348) WILSON, F. N., P. S. BARKER, F. D. JOHNSTON, I. G. W. HILL AND G. GROUT. The electrocardiogram in the earlier stages of experimental myocardial infarction. J. Clin. Investigation **12:** 993, 1933.

(349) WILSON, F. N., I. G. W. HILL AND F. D. JOHNSTON. The form of the electrocardiogram in experimental myocardial infarction. Am. Heart J. **9:** 596, 1934.

(350) WILSON, F. N., I. G. W. HILL AND F. D. JOHNSTON. The form of the electrocardiogram in experimental myocardial infarction. III. Am. Heart J. **10:** 903, 1935.

(351) WILSON, F. N. AND F. D. JOHNSTON. The vectorcardiogram. Am. Heart J. **16:** 14, 1938.

(352) WILSON, F. N., F. D. JOHNSTON, I. G. W. HILL AND G. GROUT. Form of the electrocardiogram in experimental myocardial infarction. Proc. Soc. Exper. Biol. and Med. **30:** 799, 1933.

(353) WILSON, F. N., F. D. JOHNSTON AND I. G. W. HILL. The form of the electrocardiogram in experimental myocardial infarction. IV. Am. Heart J. **10:** 1025, 1935.

(354) WILSON, F. N., F. D. JOHNSTON AND P. S. BARKER. The use of the cathode-ray oscillograph in the study of the monocardiogram. J. Clin. Investigation **16:** 664, 1937.

(355) WILSON, F. N., F. D. JOHNSTON, A. G. MACLEOD AND P. S. BARKER. Electrocardiograms that represent the potential variations of a single electrode. Am. Heart J. **9:** 447, 1934.

(356) WILSON, F. N., F. D. JOHNSTON, F. F. ROSENBAUM AND P. S. BARKER. On Ein-

thoven's triangle, the theory of unipolar electrocardiographic leads, and the interpretation of the precordial electrocardiogram. Am. Heart J. **32:** 277, 1946.

(357) Wilson, F. N., F. D. Johnston, F. F. Rosenbaum, H. Erlanger, C. E. Kossmann, H. H. Hecht, N. Cotrim, R. M. de Oliveira, R. Scarsi and P. S. Barker. The precordial electrocardiogram. Am. Heart J. **27:** 19, 1944.

(358) Wilson, F. N., A. G. Macleod and P. S. Barker. The form of the electrocardiogram. II. The character of the excitation wave in auricular muscle. Proc. Soc. Exper. Biol. and Med. **27:** 588, 1930.

(359) Wilson, F. N., A. G. Macleod and P. S. Barker. The form of the electrocardiogram. III. Opposed potential difference. Proc. Soc. Exper. Biol. and Med. **27:** 589, 1930.

(360) Wilson, F. N., A. G. Macleod and P. S. Barker. The form of the electrocardiogram. IV. The mean electrical axis and the center of stimulation. Proc. Soc. Exper. Biol. and Med. **27:** 590, 1930.

(361) Wilson, F. N., A. G. Macleod and P. S. Barker. The interpretation of the initial deflections of the ventricular complex of the electrocardiogram. Am. Heart J. **6:** 637, 1931.

(362) Wilson, F. N., A. G. Macleod and P. S. Barker. The accuracy of Einthoven's equation and the potential variations produced by the heart beat at the apices of Einthoven's triangle. Am. Heart J. **7:** 203, 1931.

(363) Wilson, F. N., A. G. Macleod and P. S. Barker. Potential variations produced by the heart beat at the apices of Einthoven's triangle. Am. Heart J. **7:** 207, 1931.

(364) Wilson, F. N., A. G. Macleod and P. S. Barker. The order of ventricular excitation in human bundle-branch block. Am. Heart J. **7:** 305, 1932.

(365) Wilson, F. N., A. G. Macleod and P. S. Barker. Electrocardiographic leads which record potential variations produced by the heart beat at a single point. Proc. Soc. Exper. Biol. and Med. **29:** 1011, 1932.

(366) Wilson, F. N., A. G. Macleod and P. S. Barker. The distribution of the action currents produced by heart muscle and other excitable tissues immersed in extensive conducting media. J. Gen. Physiol. **16:** 423, 1933.

(367) Wilson, F. N., A. G. Macleod and P. S. Barker. The distribution of the currents of action and of injury displayed by heart muscle and other excitable tissue. Univ. Michigan Press, Ann Arbor, 1933.

(368) Wilson, F. N., A. G. Macleod, P. S. Barker and F. D. Johnston. The determination and the significance of the areas of the ventricular deflections of the electrocardiogram. Am Heart J. **10:** 46, 1934.

(369) Wilson, F. N., A. G. Macleod, F. D. Johnston and I. G. W. Hill. Monophasic electrical response produced by the contraction of injured heart muscle. Proc. Soc. Exper. Biol. and Med. **30:** 797, 1933.

(370) Wilson, F. N., S. W. Wishart and G. R. Herrmann. Factors influencing distribution of potential differences, produced by heart-beat, at surface of body. Proc. Soc. Exper. Biol. and Med. **23:** 276, 1926.

(371) Wolferth, C. C. and M. M. Livezey. A study of methods of making so-called unipolar electrocardiograms. Am. Heart J. **27:** 764, 1944.

(372) Wolferth, C. C., M. M. Livezey and F. C. Wood. The relationships of lead I, chest leads from the C_3, C_4 and C_5 positions, and certain leads made from each shoulder region: the bearing of these observations upon the Einthoven equilateral triangle hypothesis and upon the formation of lead I. Am. Heart J. **21:** 215, 1941.

(373) Wolferth, C. C., M. M. Livezey and F. C. Wood. Studies on the distribution of a potential of ventricular origin. Am. J. Med. Sci. **205:** 154, 1943.

(374) Wolferth, C. C., M. M. Livezey and F. C. Wood. Distribution of the patterns of ventricular potential which determine the forms and significance of electrocardiograms. Am. J. Med. Sci. **205:** 469, 1943.

(375) Wolferth, C. C., S. Bellet, M. M. Livezey and F. D. Murphy. Negative displacement of the RS-T segment in the electrocardiogram and its relationship to positive displacement. Am. Heart J. **29:** 220, 1945.

(376) Wolferth, C. C. and F. C. Wood. The prediction of differences between precordial leads CR, CL, and CF based on limb lead findings. Am. Heart J. **20:** 12, 1940.

(377) Wood, F. C., C. C. Wolferth and G. D. Geckeler. Histologic demonstration of accessory muscular connections between auricle and ventricle in a case of short P-R interval and prolonged QRS complex. Am. Heart J. **25:** 454, 1943.

(378) Yater, W. M., V. H. Cornell and T. Claytor. Auriculoventricular block due to bilateral bundle branch lesions. Arch. Int. Med. **57:** 132, 1936.

(379) Yater, W. M. Pathogenesis of bundle branch block. Arch. Int. Med. **62:** 1, 1938.

(380) v. Zarday, I. Ekg-Axonometer. Ein neuer Apparat zur Bestimmung der elektrischen Achsen des Herzens. Klin. Wchnschr. **19:** 1084, 1940.

(381) v. Zarday, I. Bedeutung der elektrischen Momentanachsen des Herzens. Arch. f. Kreislauff. **7:** 223, 1940.

(382) v. Zarday, I. Das Elektro-Kardio-Axonogramm. Ztschr. klin. Med. **140:** 514, 1942.

(383) v. Zarday, I. Ein Beitrag zur Lösung des Vektordiagramms. Ztschr. f. Kreislauff. **35:** 18, 1943.

(384) Zeisler, E. B. A critique of Einthoven's law in electrocardiography. Proc. Soc. Exper. Biol. and Med. **28:** 12, 1930.

(385) Zeisler, E. B. and L. N. Katz. Studies of the electrical field of the heart. I. Invariants of the electrocardiogram. Am. Heart J. **8:** 676, 1933.

INTERCELLULAR CEMENT AND CAPILLARY PERMEABILITY

ROBERT CHAMBERS AND B. W. ZWEIFACH[1]

Department of Biology, Washington Square College of Arts and Sciences, New York University, New York

The passage of materials across a membrane consisting of cells, e.g., the capillary wall, requires a consideration of several structural and environmental factors. Environmental effects may be mediated through chemical action on the structure of the membrane; or they may be mechanical through the exertion of pressure on the membrane. The effects of the mechanical factor may be purely a matter of degree, variations in the amount of material passed through in a given time occurring without changing the permeability characteristics of the membrane; or they may be qualitative, distending and thereby increasing the porosity of the membrane. Even when considering the interrelations of purely extrinsic factors such as hydrostatic and osmotic pressure, it is necessary to take into account both qualitative and quantitative effects on the fluid-exchange across the membrane. The action of specific chemical substances obviously involves qualitative changes. In this regard an understanding of the structural components of the membrane is of prime importance. Moreover, since we are dealing with a hemodynamic system, we must treat the permeability of the membrane in relation to the multiplicity of factors which control the peripheral distribution of the blood.

On the basis of structural factors conditioning the passage of material across a cellular membrane we may distinguish three types (1). The permeability of one depends entirely on the cells constituting the membrane. This type is exemplified by secretory epithelium in which the constituent cells perform work in selectively absorbing material from the environment on the one side, passing it through the cytoplasm, and expelling it to the environment on the other side of the cell. An example of this kind is the wall of the renal proximal tubule in which a one-way permeability is so pronounced that even water is driven through in one direction against an osmotic pressure gradient (2). The hydrostatic pressure developed within such a tubule may be so high that the tubule distends and the constituent cells become compressed from their normal cuboidal to a flat pavement shape. Moreover, this can occur without affecting the one-way, selective permeability of the membrane.

Any cellular membrane which depends for its permeability on its constituent cells must be highly selective. The life of a cell is conditioned by its ability to maintain a differential between the environment of the cell and its interior. Concerning the penetration of substances into and their escape out of an individual cell, much investigation has been done and speculation advanced. But even the speculations have never gone so far as to assume that a cell can serve as

[1] Present address: Dept. of Medicine, Cornell University Medical College, New York, New York.

an indiscriminate thoroughfare for all material existing within certain critical dimensions of size. If the passage of materials into a cell, through its cytoplasm and out to the other side, is a sole property of colloidal and hydrostatic pressure differences, then our whole concept concerning cell-life falls to the ground.

The second type of cellular membrane has a permeability in which both the constituent cells and the intercellular material take part. Hoeber (3) has presented evidence for such an occurrence in the intestinal mucosa. In this type, the secretory activity of the cellular constituents predominates so that the composition of the fluid passed through the membrane is a mixture of secretion and intercellular diffusion products.

The third type of cellular membrane is one in which the permeability depends primarily on the intercellular cement-substance. The rôle of the cells would then be that of building blocks and be responsible mainly for elaborating the cement which lies between them and holds them together.

The relatively undiscriminating nature of the permeability of the wall of the blood capillary classifies the endothelium as a cellular membrane of this third type.[2] In other words, the permeability rôle of the constituent cells would be decidedly subordinate to that of the intercellular substance, otherwise the endothelial cell would have to be so porous as to make it difficult to conceive how such a body of protoplasm can maintain the highly specific attributes of a living cell (cf. Krogh (4)).

The mere fact of the thinness of the endothelial cell does not necessarily indicate that it possesses the wide range of permeability of the capillary wall. Layers of living protoplasm as thin as the endothelium possess a high degree of selectivity in their permeability to substances in solution. For example, the protoplast surrounding the vacuole of a marine plant cell, Valonia, is only a few microns thick, nevertheless it maintains a pronounced degree of ionic difference between the fluid in the vacuole and the surrounding medium.

For water and for nonelectrolytes of sufficiently small dimensions, e.g., CO_2 and O_2, passage into and through a cell may occur by physical diffusion depending on concentration gradients and differences of osmotic and hydrostatic forces on the two opposite sides of the cell. On the other hand, the cellular transport of electrolytes has been recognized only in secreting cells through a mechanism involving expenditure of energy beyond the basal requirements for vital integrity. The endothelial cell may serve in the physical transfer of water and presumably of dissolved gases but the permeability of the endothelial wall for substances such as all electrolytes, large nonelectrolytes and colloidal suspensions is, except

[2] There are special instances when the composition of tissue or body fluids in a particular regional area cannot be explained by simple ultra-filtration through the wall of blood vessels, e.g., synovial fluid, aqueous humor, cerebro-spinal fluid, extracellular fluid of brain tissue, etc. In these cases the barrier between the blood and the particular body cavity is double, consisting of an endothelial covered by an epithelial layer. Since the evidence overwhelmingly indicates that the endothelial layer acts as a simple ultrafilter, a secretory mechanism of the epithelial layer is probably responsible for the selective permeability of the barrier.

for particle size, so non-selective that the cell physiologist would prefer to relegate their passage to some constituent of the wall other than the living endothelial cells.

The alternative is the unlikely occurrence of active uptake by cells through a complex process of phagocytosis on an ultra-microscopic scale. The uptake by living cells of particulate matter is well recognized as phagocytosis. This process of ingestion has been extended to the uptake of colloidal dyes by means of ultramicroscopic phagocytosis. On the other hand, the only known mechanism for the elimination of such particles is either by a dissolution of the cell containing the particles or by a pinching-off of a bleb of cytoplasm enclosing one or more particles. This must involve expenditure of energy and a complex mechanism. It would be highly uneconomical for endothelial cells to use this method routinely in transferring particles from the lumen of the blood vessel to the outside. Florey (5) injected starch grains into the blood stream and found some lying in the endothelium and others outside. His claim for their passage through the endothelial cell is by inference only. Field and Drinker (6) observed the passage of calcite particles through the endothelial wall. The slowness of the passage led them to postulate uptake by the endothelial cell from the blood and ejection on the other side of the wall. However, passage through spaces in a cement substance, which presumably is sticky, may also be slow. Moreover, the physical consistency of the cement substance between the cells must be such as to permit the passage of particles of colloidal size up to aggregates and even leucocytes and red blood cells.

The material between the cells can act as a physical filter without the expenditure of energy, a mode of filtration stressed by Landis (11). Such a filter could exhibit variations in its limiting porosity of vessels in different tissues and also in the same tissue under different environmental conditions. Likewise, the findings of Abell (7) that newly developing blood capillaries are more highly permeable than later, suggest the gradual development of an interendothelial cement. Wilbrandt (8) cites the limiting porosity of the capillaries in the glomerular tuft of the kidney to be that of a molecular volume of 68,000. The uppermost limit of porosity of a cell membrane has never been claimed to be in excess of 342, the molecular volume of saccharose. A route other than the cell proper should be postulated in order to account for the passage, in bulk, of molecular aggregates through the endothelial wall.

The term, capillary permeability, has been loosely used without discriminating between those phenomena which are indirect in their action, i.e., through forces in the blood and surrounding tissues which do not affect the intrinsic property of the membrane barrier itself, and those which are direct, i.e., through factors which induce actual changes in the composition of the capillary wall.

A search of the literature reveals the lack of clear-cut evidence for defining the type of permeability referable to the changes being discussed. Many of the fluctuations in tissue-blood exchange which have been described as evidence for altered capillary permeability may not involve actual changes in the structure of the capillary wall. Such fluctuations are to be attributed rather to extrinsic

phenomena, such as fluctuations in hydrostatic pressure, factors regulating peripheral blood flow, avidity of extra-capillary tissue for either solute or solvent, under normal or experimentally induced conditions, etc. Alterations in permeability to be ascribed to changes in the membrane would be of primary importance in pathological phenomena. Under normal conditions it is highly probable that factors exist which maintain the capillary wall in a stable physico-chemical state. An elucidation of these factors must distinguish between effects on different components of the capillary wall.

ARCHITECTURE OF THE CAPILLARY WALL. There is good evidence for the existence of three structural components of the capillary wall, variations of each of which may alter conditions affecting the passage of materials through the wall. These are: the endothelium *per se*, consisting of pavement-like cells, and an intercellular cement; an endocapillary lining, which is non-cellular and is possibly derived from the circulating blood proteins; and third, a pericapillary sheath, which serves as an outer, supporting layer with characteristics common to the surrounding connective tissue matrix.

1. *The Capillary Endothelium.* The outstanding component of the capillary wall is the endothelium proper, the barrier usually considered as regulating the passage of materials from blood to the surrounding tissue. Most discussions assume that the exchange across the endothelial membrane involves the endothelial cells and Danielli (9) has gone so far as to postulate a continuous plasma membrane as the conditioning factor in capillary permeability. A more likely alternative, based on the highly porous character of the capillary wall, is to relegate a prominent rôle to the intercellular cement acting as physical ultrafilter.

Insistence on the cell as the primary route for tissue-blood fluid exchange would necessitate considering the endothelial cell as a radically modified, parchment-like cell remnant, an unwarranted assumption since the endothelial cell is known to possess fundamental properties of a living cell. It is capable of changing its form and size; it possesses tone; it is irritable and reacts to prodding; it undergoes mitotic division and, in common with living cells, its nucleus is a prominent structure which can be stained with basic dyes only after death. The concept that the endothelial cell plays a subordinate rôle in the exchange across the capillary wall does not imply that the cells are not vitally concerned in maintaining the capillary wall as a living structure. Aside from their functions in repair and regeneration there is evidence (10) that the endothelial cell elaborates the cement substance which binds the cells into a continuous membrane.

The concept of capillary permeability presented in this review is based on the properties of the non-cellular components of the capillary wall. The intercellular cement substance serves as a filter, the selective properties of which depend upon variations in its porosity. The passage of material through such a membrane would depend upon the number, size, shape, distribution and other properties of the pores of the cement also upon the nature of their coating and the extent to which the pores can be plugged.

A quantitative consideration of the passage of substances through the capil-

lary wall must take into account the surface area of the cement relative to that of the cells available for filtration. Landis (11) cites figures to show that the filtration rate across the capillary wall occurs at 3.7×10^{-2} cc./cm.^{2}/min./atmos. This is approximately 100 times the value for the permeability to water of the arbacia egg as computed by Lucké. Wilbrandt (cf. 8) made a comparison of the absolute water permeability of red blood cells, leucocytes, fibroblasts, arbacia eggs and amoebae with that of a blood capillary wall. On the basis of equal pressure, area and time, he arrived at the same value as that of Landis, i.e., the permeability of the blood capillary to be 100× that of a living cell. Let us assume that the endothelial wall is 99 per cent cellular and 1.0 per cent intercellular and let us also assume that the permeability of the individual endothelial cell is the same as that of cells which have been measured. Then in 1 sq. cm. of capillary wall we would have 0.01 sq. cm. of intercellular cement having a high permeability and 0.99 sq. cm. surface of the cellular constituent having a low permeability. The permeability constant as found by Landis could refer to only the one hundredth part of the square centimeter wall consisting of cement substance. Let us imagine 1 sq. cm. of wall consisting entirely of the cement substance. The permeability constant of this 1 sq. cm. area would be larger by a value of 4 orders of magnitude than a comparable area consisting only of cells. Thus, any slight change in the nature of the cement should enormously affect the permeability of the endothelial wall. The pore size in the cement may vary under different conditions, e.g., mechanical stretching of the membrane or by changes in the chemical nature and pH of surrounding medium. Such changes may influence the amount of fluid which passes in a given time. Furthermore, a mere change in shape of the pores from an elliptical to a circular, will change the limiting porosity of the membrane to colloidal constituents without affecting the rate of water exchange (12).

Intercellular cement. In view of the relative importance of the inter-endothelial cement it is necessary to discuss factors concerned with the maintenance of its physical state. The early investigators, Ringer, Herbst, Overton, stressed the existence of a reversible, organic calcium salt which serves as a cohesive substance for binding cells together. The physical state of the cement can be varied by changing either the pH or the calcium content of the medium (cf. 10). This non-cellular constituent of the capillary wall was recognized by Cohnheim in his earlier papers about 1867 and by Arnold in 1887 who claimed that a loosening of the cement substance may become so great as to result in the formation of stigmata through which diapedesis occurred. Rabl, in 1893, suggested that the inter-endothelial lines, made visible with silver nitrate, were due to the formation of a silver proteinate.

The capillary circulation in the frog's mesentery has proven to be an excellent region for experimental studies on the rôle of the interendothelial cement (cf. 10). Artificial perfusates containing ash-free gelatin were used, the calcium content and pH of which were varied within viable limits. The gelatin was used since without it the Ringer's solution, even with the normal complement of calcium and

at the blood pH of 7.4, soon resulted in excessive edema indicating an abnormal permeability of the capillaries.

Changes in the physical state of the inter-endothelial cement could be detected by the adhesiveness to it of carbon particles suspended in the circulating perfusate. An increased sticking indicated a softening of the cement. Adhesiveness of India ink to the endothelium has generally been taken, e.g. by Landis, to indicate abnormal increase of permeability. Under normal conditions only occasional particles of carbon adhere to the vessel wall. When the acidity of the perfusate was increased or its calcium content was decreased there occurred an increased sticking and accumulation of the carbon particles especially along the inter-endothelial lines of the capillary wall. The softening of the cement was accompanied by a marked edema. The accumulation of the carbon along the endothelial lines indicated where the outward filtration of fluid was most pronounced. When excess calcium was present no such accumulation of carbon occurred along the inter-endothelial lines; moreover, no edema developed.

Under normal conditions the cement is being continually replaced. This was graphically demonstrated by applying a small quantity of 10 per cent silver nitrate with a micropipette to the surface of a capillary. The inter-endothelial lines immediately blackened and, as the blood flow continued, a blackened, amorphous material dropped off into the stream. The absence of any sign of leakiness or visible alteration in the flow indicated a continuous replacement of the cement as it was washed away. Micro-trauma invariably is accompanied by increased leakiness in the affected area. With more severe trauma a striking phenomenon is the appearance of transparent, glutinous masses exuding from the inner wall. These masses, made obvious by the carbon sticking to them, slough off and are carried downstream. The increased porosity of the vessel wall is indicated by a slowing of the blood stream as it approaches and passes by the affected region. In the region of sluggish flow the red cells become crowded together. After a few minutes the normal rate of flow returns with a recovery of the capillary wall to its former normal state. The reversibility of the phenomenon, from the development of a leaky state to eventual recovery, indicates a reconstitution of the cement which had been lost following the initial injury.

The suggestion is offered that an important rôle of the endothelial cell is the elaboration of an intercellular cement, the chemical stability and reactivity of which control the permeability of the blood capillary.

2. *Endocapillary Layer.* Another component of the capillary wall is a thin, non-cellular layer lining the inner surface of the endothelium. Its formation has been ascribed to the adsorption of a blood protein (13).

Danielli, in studies on perfused hind-legs of the frog, has offered indirect evidence for an adsorbed protein layer based on differences in the porosity of the capillary wall which occur with variations in the colloidal content of the perfusion fluid. He used a variety of colloids in isosmotic concentrations and found the order of relative efficiency in preventing edema, i.e., reducing capillary permeability to be: serum > acacia > ovalbumin > hemoglobin. He postulated that

certain blood proteins have a specific tendency to be adsorbed on the walls of the capillary pores and suggested that the particle size of the colloidal constituents play an important rôle. Such an adsorption mechanism would explain the findings of Krogh and Harrop (14) and of Drinker (15) that the edema caused by a perfusate of 3 per cent gum acacia can be markedly reduced by the addition of small amounts, e.g., 10 per cent, of blood serum.

Danielli also brought up the interesting point that certain basic proteins, e.g., clupein, added to serum, cause an increase in capillary permeability when the perfusate is kept at a constant pH. He suggested that the increased permeability is due to a displacement of the adsorbed plasma protein layer by the more highly surface-active clupein which forms a much thinner layer than does plasma protein.

In our investigations on the action of calcium and of pH on the inter-endothelial cement we were struck with the fact that perfusion fluids consisting of purely crystalloidal solutions, even with adequate calcium and proper pH, induced leakiness of the capillary wall with consequent edema although there was no indication of deficient inter-endothelial cement. This suggested that in the absence of a colloid the cement filter, although intact, lacks a physical component which is furnished by a colloid. The cement filter is porous enough to permit passage of colloidal material. A change in porosity occurs with the addition of slight amounts of a colloid which probably clogs the cement filter, thereby diminishing its porosity. Evidently, the adsorbed colloid constitutes the endocapillary layer. We used various colloids and found their relative efficiency in causing a recovery of a leaky capillary to be of the order: blood serum > bovine albumin > ash-free gelatin > gum acacia. This accords with similar findings of Danielli in relation to perfused blood vessels.

Direct microscopic evidence for such a layer has not been forthcoming. A near approach has been from observations of the frog's mesentery perfused with a crystalloid, frog-Ringer's solution containing Evans blue which is known to combine with blood albumin. During the perfusion thin strands and sheets of a faintly colored blue, translucent material were seen sloughing off the inner surface of the capillary and being carried away in the stream. This sloughing continued for 10 to 15 minutes during which the mesentery became increasingly edematous. Changing the perfusate to one containing either 1 per cent gelatin or 10 to 20 per cent frog or fowl serum resulted in an appreciable reduction of the edema. When the perfusion fluid was changed back to the original crystalloidal solution with Evans blue there again appeared the sloughing off of faintly colored sheets of glutinous material.

3. *Pericapillary Sheath.* The existence of a supporting tissue investing blood capillaries has been suggested by several investigators, e.g., Heimberger (16), Benninghof (17), Midsumo (18) and Michels (19). Volterra (20a) made extended studies on this subject and has clearly demonstrated the presence of a closely fitting, investing layer of argentophil fibrils over the outer surface of the endothelium of capillaries. Such an investment is well exhibited in brain tissue.

In other tissues it appears to be continuous with argentophil fibrils surrounding glandular alveoles, muscle fibers, nerves, etc.

Volterra obtained variations in the amount of blood flowing through a given tissue to be in accordance with changes in pH and electrolytic content of the perfusate. He ascribed this to differences in action of the perfusate on the pericapillary sheath thereby conditioning the size of the vessels in the capillary bed. The significance of Volterra's conclusions lies in his conviction that variations in the amount of the circulating fluid may be referred to the supporting tissue without the necessity of assuming changes in the endothelium proper. This conclusion was not verified by looking for actual changes in the capillary structure.

In an earlier paper (20b) he describes, in cases of nephrosclerosis and hypertension, a transformation of the pericapillary sheath from the normal delicate layer containing finely anastomosing argyrophilic fibrils into a thick layer which may be hyalinized. Under such conditions he observed the presence of red blood cells permeating the wall and believed this to indicate an increased porosity of the vessel. More recently, Gorev and Smirnova-Zamkova (21) described similar changes, in cases of edema and hypertension, in a layer which they termed a hematoparenchymous barrier. The existence of a pericapillary sheath and its possible rôle in various pathological conditions makes a study of the normal properties of the sheath one of prime importance.

The presence of a relatively stiff layer of material against the outer surface of the capillary endothelium is evident when a white blood cell is undergoing diapedesis. The extruded portion of the diapedesing cell never moves directly away from the wall but spreads over the outer surface of the endothelium and remains for some time pressed closely between it and the pericapillary sheath. The cell finally works its way through the interstices of the sheath to move more freely in the less resistant regions of the connective tissue matrix. The relation of this sheath to the connective tissue is suggested by the fact that agents which affect the connective tissue matrix appear to exert a similar action on this pericapillary sheath. We have made intravenous injections of the mucolytic enzyme, hyaluronidase,[3] and also have applied solutions of it with micropipettes on the surface of capillaries in the mesentery of the frog. In both cases there was no evidence of increased stickiness of the capillary wall or of any increase in permeability. A significant feature which did occur was the abrupt development of microscopic petechial hemorrhages. These occur through spots in the wall weakened by the softening of the supporting connective tissue sheath and, therefore, easily ruptured by the internal blood pressure. We regard hyaluronidase as a factor in accentuating capillary fragility rather than in inducing direct changes in capillary permeability (22).

Moreover, we found that bacterial toxins[4] (Shiga exotoxin, Clostridium

[3] The hyaluronidase was prepared from testicular extracts and supplied through the courtesy of Dr. Karl Meyer of the College of Physicians and Surgeons, Columbia University, N. Y.

[4] These bacterial toxins were supplied through the courtesy of Dr. René Dubos of the Rockefeller Institute and Dr. Colin MacLeod of New York University College of Medicine.

welchii, C. perfringens and C. septicum), injected into the blood stream of rats, cats and dogs, caused the production, several hours later, of petechial hemorrhages among the mesenteric and omental capillaries with no evident dissolution of the inter-endothelial cement. These bacterial toxins are known (23) to contain a hyaluronidase component to which the increased fragility of the capillaries can be attributed. We therefore regard Duran-Reynal's (cf. 22) postulate that hyaluronidase regulates capillary permeability as being concerned with an increase in fragility of the capillary wall rather than with the more readily reversible phenomenon of changes in capillary permeability.

4. *Micromanipulative Studies on the Capillary Wall.* The structural make-up of the normal capillary wall is so delicate that mechanical handling even on a micrurgical scale invariably produces changes in its permeability characteristics. This has already been indicated by Landis (cf. 11). The reversibility of such an injury depends upon the intensity of the change produced. Merely moving the tip of a microneedle back and forth in the close vicinity of a capillary will inflict an evanescent injury sufficient to cause an outflow of fluid. This is made evident by an approach, to the injured spot, of red cells and an outward leakage of dye. In mammals there also occurs a temporary sticking of platelets to the inner surface of the capillary wall. A more drastic reaction, but one which is still reversible, can be occasioned by gently rubbing the surface of the capillary with the blunt tip of a microneedle. This induces not only a sticking of platelets and of leucocytes but also a rapid escape of dyes of relatively large colloidal dimensions, such as Evans blue. The leakiness of the wall may become so high that the red cells are diverted from their forward course and applied against the inner surface of the vessel. With still more severe injury but insufficient to cause actual rupture, red cells may be passed through minute openings to the outside. The various effects, grading from a slightly increased porosity to a pronounced leakiness, are similar to those observed when perfusion fluids are used which are deficient in calcium or are more acid than normal. Similar effects are obtained when the adjacent tissue is acidified. Evidence of this has been obtained (24) by depositing a microdrop of olive oil containing glacial acetic acid in the vicinity of a capillary.

All the procedures, viz., microtrauma, use of calcium-deficient perfusates, calcium-containing, acid perfusates, or local acidification, act by softening (made evident by carbon sticking) and dissolving the inter-endothelial cement. The changes in the cement caused by the microtrauma are possibly due to a local production of an "acid of injury". An additional factor is the contracting reaction of individual endothelial cells. Contiguous cells can be made to withdraw from one another sufficiently to loosen the cement between them. An extreme example of the latter type of change is the shrinkage reaction of the endothelial cells to hypertonic sucrose or mannitol solutions locally applied with a micropipette. The cells retract from one another to the extent that spaces between them become studded with dumb-bell shaped red cells partly extruded through the vessel wall. As the hypertonicity passes off the endothelial cells return to their normal expanded condition and close the spaces

between them. The red cells, caught in the wall, then either move out, fall back into the vessel, or become pinched in two (25).

SUMMATION ON SIGNIFICANCE OF ARCHITECTURE OF CAPILLARY WALL. The preceding analysis of the constitution of the capillary wall presents a structural basis concerned with the interchange of material between blood and tissue. The inter-endothelial cement serves as the basic framework, the porous interstices of which vary with changes in the electrolytic balance of the medium. The endocapillary lining appears to be an adsorbed layer of a blood protein which penetrates the interstices of the cement and serves further to regulate the pore size of the cement filter. The third constituent is the pericapillary sheath which appears to be a condensation of connective tissue serving to give mechanical support to the blood capillary. It is probably sufficiently porous to allow free passage for the fluid part of the blood.

Each of the above three components can be affected separately by a variety of conditions so as to induce changes in the permeability of the wall. Hence it is difficult to assume a single permeability factor, such as a permeability hormone or vitamin, to account for the overall maintenance of fluid balance between blood and tissue.

It is to be noted that variations in the permeability of the capillary can be accounted for by changes in the non-living components of the capillary wall. An important function of the endothelial cells appears to be the elaboration of the cement. In extreme cases, since they are irritable, the cells can contract or expand and in this way affect the porosity of the wall by changing the physical characteristics of the cement filter.

TOPOGRAPHY OF THE CAPILLARY BED. Since capillary permeability is intimately related to hemodynamic aspects of the peripheral circulation, it is necessary to review briefly the factors concerned in the distribution of blood through the capillary bed. It is unfortunate that an understanding of the actual anatomical topography of the capillary bed has been generally overlooked in attempts to elucidate its functional activities. The tendency has been to consider the capillaries as simple tubes with the only anatomical differentiation of classifying them as arterial or venous.

Topographical studies of the capillary bed have shown that the vascular components may be of different sorts which are precisely arranged into organized units of structure and function (26). In some tissues the capillaries have no discernible organization. This type is found in tissues which maintain a fairly constant level of flow volume. In other tissues, where nutritive demands vary with changes in functional activity, a well defined pattern exists, e.g., in the muscular system and in the gastro-intestinal tract. During periods when the tissue is inactive the flow is restricted to preferential channels, while during periods of increased activity the flow becomes widespread throughout the capillary network.

The preferential vessels have been termed thoroughfare or a-v channels. The proximal portion of these channels, termed metarterioles, together with their precapillary sphincteric offshoots, are muscular and spontaneously undergo

periodic changes in caliber. This type of movement has been termed vasomotion, a slow intermittency of partial relaxation and constriction at intervals of about 30 seconds to 3 minutes. The vasomotion serves as a mechanism for restricting the blood flow to the thoroughfare channels or permitting it to become more widespread through the capillary bed. It is the mechanism which locally adjusts the peripheral flow to the needs of the tissue. The precapillary sphincteric offshoots lead into an inter-anastomosing system of true capillaries (devoid of muscular elements) which constitutes the bulk of the bed. The capillaries rejoin the distal continuation of the thoroughfare channels through inflowing tributaries.

The hydrostatic pressure in any of the true capillaries throughout the bed is extremely variable. It depends from moment to moment on the intermittent state of the precapillary sphincters and on the efficiency of the venous outflow. On the other hand, the thoroughfare channels maintain a proportionally constant pressure relationship between their arteriolar and venous ends. This condition depends largely upon the pressure in the arterioles leading into them. The venous outflow from the bed is determined by the rate and magnitude of the flow through the thoroughfare channel.

Thus, the thoroughfare channel is the basic structural and functional component of the capillary beds. The capillary network is accessory to it and exhibits great variability in its rôle in fluid exchange. This variability was noted by Landis and is reflected in the high degree of scattering exhibited in recorded measurements on intracapillary pressure (Landis (27)). Such measurements would be of greater significance and more consistent if they were made on the arterial and venous segments of the thoroughfare channels.

The relatively low hydrostatic pressure in the true capillaries throughout the bed is accomplished by the resistance offered through the sharp, backward twisting of the junctional portions of the outflowing precapillary offshoots, together with the relatively wide postcapillaries which lead into the distal portion of the thoroughfare channel. The sphincteric action of the precapillary musculature is a further factor in sharply lowering the arterial pressure as the blood enters the true capillaries.

The flow through the thoroughfare channels is usually distinctly more rapid than elsewhere in the bed, and the fall in hydrostatic pressure from their arteriolar to their venous ends must be of lesser magnitude than that which occurs in the side branches. As a result, the channel is to be considered as the predominating region for outward filtration while inward filtration would be a characteristic function of the true capillaries. The occurrence of outward and inward filtration at different sites makes it unnecessary to assume that the movement of fluid and of suspended materials should occur simultaneously in opposite directions through the wall of the same capillary vessel.

VASOMOTION AND ITS INFLUENCE ON FLUID EXCHANGE[5]. Vasomotion is the

[5] This and other sections constitute an amplification and an analysis of accumulated observations from a group study on the capillary circulation in secondary shock by the authors with E. L. Chambers, G. H. A. Clowes, G. W. Duncan, C. G. Grand, C. Hyman, M. J. Kopac,

term applied to the spontaneously occurring periodic relaxation and constriction of the thoroughfare channel and of its precapillaries (cf. 10). The dilator and constrictor phases of the vasomotion alternate irregularly from minute to minute, and the duration of one or the other phase varies under different physiological conditions. The vasomotion of the muscular, proximal portion (the metarteriole) of the thoroughfare channels is wavelike and exerts a milking action on the inflowing postcapillaries which lead into the distal portion of the channel. Normally, the blood-flow through the channel is fairly constant as a result of which its hydrostatic pressure should remain relatively high. The vasomotion of the precapillary offshoots, manifested by an opening and closing of their sphincters, conditions the flow through the true capillaries among which it occasions alternate periods of varying hydrostatic pressure. The relative duration of these periods varies greatly under different conditions. The fluid exchange in the capillary bed is thus greatly influenced by the character of the vasomotion. This delicately balanced activity depends upon a nervous factor, which is vaso-excitor, and on humoral factors which may be either vaso-excitor or vasodepressor. The tissue origin of some of the humoral factors offers a means for the vasomotion to act as a mechanism for locally regulating the distribution of the blood and, thereby, the extent and duration of inward and of outward filtration in the capillary bed.

The direct action of the vasomotion affects the fluid exchange by determining the flow of blood to be either by way of the thoroughfare channels or of the true capillaries. The fluid exchange is also affected indirectly by the influence which the distribution has on the rate of venous outflow from the capillary bed. For example, when vasomotion is deficient or absent, the precapillaries remain open and the propelling force of the pressure transmitted by the arterioles becomes dissipated through the numerous capillaries of the bed. This spread of flow causes a slowing in the rate of overall movement through both capillaries and thoroughfare channels with the result that blood tends to accumulate in the collecting venules. This induces sufficient back pressure to favor an overall outward filtration. On the other hand, when vasomotion is active, the flow through the capillary bed is increasingly restricted to the preferential channels so that the propelling force of the arterial pressure through them is sufficient to enhance a rapid flow in the collecting venules. The rapid venous flow favors drainage into the venules from the true capillaries in which inward filtration is thereby accentuated.

We may recapitulate the influence of vasomotion on the effectiveness of the hydrostatic and of the colloid osmotic pressures in the different regions of the capillary bed. Variations in vasomotion are of two sorts: first, alterations in the rate of the intermittent caliber changes of the thoroughfare channel and,

---

M. E. Krahl, R. E. Lee and B. E. Lowenstein, published and being published in the Am. J. Anat., Am. J. Physiol., Annals of Surgery, Proc. Exper. Biol. and Med., and Surgery, Gynec. and Obstetrics. Much of the work was presented in the periodic reports sent to the Committee on Medical Research of the O. S. R. D. and to the subcommittee on Aviation Medicine of the National Research Council during the years of 1941 to 1945.

second, alterations in the relative duration of the constrictor and the dilator phases of the precapillary sphincters. The most effective hydrostatic pressure occurs in the thoroughfare channel along the length of which the onward flow is fairly constant and rapid. Moreover, the rate of vasomotion of the channel determines the rate of venous outflow from the bed by its influence on the inflow from the postcapillaries which lead into its venous end.

The colloid osmotic pressure should be most effective in the network of true capillaries for absorbing fluid from the tissue. However, its effectiveness is variable and depends upon shifts in the hydrostatic pressure conditioned by the relative duration of the successive constrictor and dilator phases of the precapillary sphincters. The consequent periods of flow and of no flow in the true capillaries constitute a mechanism whereby the colloid osmotic pressure is periodically greater than the hydrostatic pressure. The existence of varying pressure conditions was inferred by McMaster (28) who demonstrated the intermittency of fluid uptake in the subcutaneous tissue by inserting a micropipette into the skin.

Finally, the hydrostatic pressure in the thoroughfare channels also depends on the vasomotor activity of the terminal arterioles outside the capillary bed. This activity of the terminal arterioles tends to maintain the hydrostatic pressure in the thoroughfare channels at a constant level despite fluctuations in the systemic blood pressure. The arterioles accomplish this by undergoing constriction when the arterial pressure rises, and by dilating when the pressure falls. Under certain conditions the arterioles can be affected, e.g., after an intravenous injection of fever-producing toxins (unpublished data). The arterioles then become dilated while no change occurs in the normal rhythm of the vasomotion of the thoroughfare channels. This phenomenon is accompanied by hemoconcentration made evident by close packing of blood cells in the collecting venules. The following mechanism is suggested for this condition. The dilated arterioles permit the blood to enter the capillary bed under an increased head of pressure. At the same time the maintenance of the normal periodicity of the vasomotion favors the flow through the thoroughfare channels. This produces an excessive outward filtration as a result of the increased pressure in the channel. On the other hand, the true capillaries maintain their normal intermittency with no corresponding increase of inward filtration. The result is that the overall balance favors outward filtration with consequent hemoconcentration.

It is also possible to have hemodilution with no change in the vasomotion. This occurs when the head of pressure entering the capillary bed is significantly reduced. Such a situation occurs immediately after the onset of bleeding in acute hemorrhage. The larger arteries and arterioles undergo widespread vasoconstriction to compensate for the suddenly reduced blood volume. The constriction also occurs in the terminal arterioles so that the effective hydrostatic pressure in the thoroughfare channels is lowered with a consequent reduction of outward filtration. However, vasomotion remains active with good venous outflow so that inward filtration is maintained at a normal rate in the true

capillaries. This shifts the balance in favor of inward filtration, resulting in hemodilution.

The above phenomena illustrate imbalances in fluid-exchange in the capillary bed with no disturbance in vasomotion, the imbalance resulting only from changes in the vasomotor activity of the terminal arterioles. There are also instances in which a change in the intensity of vasomotion alters the balance of fluid-exchange. For example, when the loss of blood during acute hemorrhage exceeds two per cent of the body weight, the vasomotion becomes accelerated with a still further accentuation of hemodilution. This can be explained as follows. As the frequency of the vasomotion increases, the duration of the constrictor phases becomes prolonged relative to the dilator phases. Concomitant with the long constrictor phases which reduce transmission of pressure into the side branches there is a progressively longer period during which the hydrostatic pressure in the true capillaries remains extremely low. As a consequence, inward filtration and tissue dehydration become still further accentuated. An additional aid in this direction is the increased frequency of the precapillary sphincteric contractions, producing a pronounced intermittency of the flow through the capillary bed. Moreover, the wavelike milking action of the thoroughfare channel facilitates drainage of the post-capillaries into the venous circulation.

We have been considering instances in which either the vasomotion or the state of contraction of the feeding arterioles can be affected separately. When both are suspended simultaneously, edema results. A cessation of vasomotion together with a dilated state of the feeding arteriole flushes the entire bed, resulting in excessive outward with little or no inward filtration.

In summary, deficiency or absence of the vasomotion shifts the balance of fluid exchange in favor of outward filtration while enhanced vasomotion shifts the balance to inward filtration. Thus, the hemodynamic relations of vasomotion and the forces set up by movement of fluid through one or another of the vascular components of the bed must be regarded as of major significance in affecting fluid exchange between the blood and tissue. It follows that some types of edema are the result of disturbances in the vasomotion mechanism and do not necessarily involve alterations in the permeability of the capillary wall.

A list of the factors which influence vasomotion is of interest in this discussion. Enhanced vasomotion occurs with acute hemorrhage, sympathetic stimulation, intravenous administration of adrenalin, of angiotonin or of adrenal cortical extract. Diminished vasomotion occurs with a rise in temperature within viable limits of 37.5 to 41°C, decrease in temperature, direct trauma, increased vital activity (muscular exercise, secretory activity, etc.), or elaboration of vasodepressor principles following prolonged anoxia. Many agents which have been found to increase capillary circulation do not necessarily depress vasomotion. Among these have been found histamine, adenylic acid, adenosine, kallikrein and acetylcholine (unpublished data).

VASOMOTION AND LYMPH FLOW. There is an intimate relation between vasomotion and the rate of lymph flow in the terminal lymphatic channels. This was studied in the mesentery of the cat, dog, mouse and rat. During the resting state of a tissue the vasomotion is active to the extent of producing relative ischemia, and little or no flow of lymph can be discerned in the terminal lymphatics. The absence of an active flow throughout the bed makes the large surface furnished by the true capillaries available for inward filtration and leaves no excess in the tissues.

On the other hand, during the active state of a tissue the vasomotion becomes diminished so that an overall, hyperemic flow is favored. This is accompanied by an appreciably increased flow of lymph in the terminal lymphatics. The prolonged periods during which the precapillary sphincters remain dilated favor outward filtration in the true capillaries. Edema is prevented only by an absorption of fluid into the lymphatic capillaries.

GRADIENT OF PERMEABILITY. Starling's (29) stimulating concept of fluid transfer between blood and tissue is undoubtedly true as an overall aspect of the phenomenon. According to this concept outward filtration occurs where the hydrostatic pressure is greater than the colloid osmotic pressure of the blood while inward filtration occurs where the conditions of pressure are reversed. Difficulties arise when one attempts to allocate specific regions in the capillary bed where outward and inward movements of fluid occur. According to Starling's postulate, the wall of the capillaries throughout a given tissue possesses uniform characteristics of permeability. Fluid transfer under physiological conditions would then depend solely on pressure relationships of hydrostatic and collloid forces.

Landis (27, 30, 31, 32, 33) in his well-known micromanipulative determinations of capillary blood pressure, found considerable variation in the values obtained. By averaging the pressure readings, he found that those from the arterial side of the bed tended to be significantly higher, and that those from the venous side of the bed were lower than the known colloid osmotic pressure of the blood. He concluded that outward filtration of fluid and its absorption through the wall of the capillaries is a function of the observed intracapillary pressure. Confirmatory findings were obtained by Gordon-Königes and Ottó (34) from their micromanipulative experiments on the capillaries of the intestinal villi of the cat. They found that the flow in the lacteal of the villus was correspondingly greater the higher the capillary blood pressure.

Landis takes the view that the preponderantly higher blood pressures obtained by him among the capillaries in the arterial regions of the bed in the frog's mesentery, indicate that outward filtration, on the average, occurs in the arterial region of the bed while absorption of fluid from the tissues occurs in the venous capillaries where the average hydrostatic pressure tends to be lower than the colloid osmotic pressure of the blood. He obtained evidence in support of this from the ingenious experiment of placing the blunt end of a micro-rod across a flowing capillary so as to occlude its lumen locally and obstruct the flow. He obtained values for the pressure in the arterial end of the capillary proximal

to the obstruction indicating that it had assumed the value of that of the vessels which had been flowing into it, while the pressure distal to the obstruction fell to a minimum and acquired a value corresponding to the vessel flowing from it. The crowding together or the moving apart of the corpuscles in the two segments of the capillary, taken by him to indicate outward or inward filtration, was consistent with the degree of pressure he recorded in relation to the known colloid osmotic pressure of the blood. The procedure of occluding the capillary with a micro-rod requires very careful technique because of the extraordinary ease with which damage may be inflicted, causing an abnormal increase in porosity of the wall in the obstructed region. Furthermore, it is obvious that the pressure conditions induced by the obstructing needle do not necessarily prevail in the same capillary when the obstructing needle is not there. We have obtained similar results with capillaries irrespective of the position of the capillary in the capillary bed provided the capillary exhibits a good flow prior to the placement of the obstructing needle.

In his 1926 paper (cf. 33) Landis distinguished between the capillaries which are in more or less direct relation with the finest arterioles and the venous capillaries which merge to form the venules. He noted particularly that the capillaries, where they spring from the arterioles, are narrow and at times exhibit "slit-like openings". He remarked on the conditions at the arteriole-capillary junction as being related to his observed "rather sharp reduction in the pulse pressure on passing from the arteriole to the capillary". The junctional regions of these capillaries are doubtless the vessels we have termed the precapillaries, possessing muscular sphincters, and which we have described as springing both from terminal arterioles and from the periodically constricting proximal portions (metarterioles) of the thoroughfare channels.

The question now arises as to the regional distribution of the vessels in the capillary bed which are concerned with the outward and those with the inward transport of fluid across their walls. Rous, Gilding and Smith (35), arguing from the more rapid escape, at the venous end of the capillary bed, of dyes introduced into the blood stream, claimed that the porosity of the vessel at the venous was greater than at the arterial end and that outward filtration also occurred there.

Landis (36) dismisses the possibility that the diffusion of dyes is always a dependable measure of the direction and rate of fluid transport since diffusion may conceivably occur in the opposite direction to fluid flow. Danielli, in support of the Starling theory, has offered the hypothetical explanation that the observed accumulation at the venous end is due to the reabsorption of fluid leaving behind whatever dye has escaped.

The suggestion is made here that the introduction of a concept regarding the capillary bed as possessing a pattern of specialized units of function built about the thoroughfare channels may bring into harmony the seemingly divergent points of view held by Landis and by Rous and his co-workers.

According to our view the thoroughfare channel is pre-eminently the site for outward filtration while the true capillaries, which branch off from the

channels (as outflowing vessels at the proximal and as inflowing at the distal end of the channel), serve largely for inward filtration especially during the resting stages of the tissue involved.

Landis himself commented on the great variability of the values in his recorded capillary pressure readings. He states that the pressures differed not only in different capillaries coming from the same arteriole but also in the same capillary from moment to moment. On the average, the more rapid the flow in a given capillary the higher was the recorded pressure. This variability may be accounted for, at least in part, by the periodic opening and closing of the precapillary sphincters. The conditions affecting this activity in ischemia and hyperemia are discussed elsewhere in this article.

The findings of Smith and Dick (37a) are of interest in this regard. They introduced into the circulation a continuous stream of glucose immediately after an intravenous injection of Chicago blue 6B, a poorly diffusible colloidal dye. They found a great increase in the plasma volume, indicating passage of fluid from the tissues to the blood. Despite the evident dehydration of the tissues, they observed that the accumulation of the dye outside the capillary blood vessels occurred in the same venous regions as described by Smith with Rous and others. They concluded that the movement of fluid from the tissues to the blood does not essentially alter the gradient of vascular permeability. Moreover, their findings have been interpreted as meaning that outward diffusion of dye and inward filtration or absorption of fluid can occur simultaneously in the same vessels.

This raises the issue regarding diffusion of a dye against a current of moving fluid. In our observations the diffusion of dye outward through the wall of the thoroughfare channel is too rapid to permit any other assumption than that the dye is being carried with the stream of the fluid in which it is suspended. In the words of McMaster and his associates (37b) concerning dyes in aqueous solution "where water goes they may be expected to go, unless their molecules are large enough to be held back selectively by the membrane".

We may now consider the alternative that the influx of fluid occurs in a region of the capillary bed other than that from which the dye was escaping. According to our findings, the inflow of fluid which makes for hemodilution occurs in the true capillaries where the hydrostatic force is generally insufficient to drive fluid out. In the case of Smith and Dick's experiments the hypertonicity of the sugar solution would increase the effectiveness of the colloid osmotic pressure of the blood in absorbing fluid into the true capillaries. At the same time the escape of dye would continue along the thoroughfare channels which are increasingly porous along their distal portions and which evidently maintain sufficient hydrostatic pressure to overcome the effect of the hypertonicity induced by the sugar solution.

The accompanying diagram (fig. 1) presents three concepts regarding fluid movement in the capillary bed.

The capillary bed is represented as a tube passing from *a*, the artery, to *v*, the vein. The arrows show the direction of blood flow and of fluid movement across the capillary wall.

X presents the concept of Rous and his associates in which the arrows only apply to the preponderancy of dye-escape at the venous end.

Y presents Starling's concept as demonstrated and amplified by Landis from his capillary blood-pressure determinations. In this, the fluid movement is predominatingly outward at the arterial end and inward at the venous end.

Our concept combines *X* and *Y*. *X* represents the thoroughfare channels in which outward filtration occurs together with the phenomenon of vasomotion which intermittently opens and closes the precapillary sphincteric offshoots. The latter lead into *Y*, the true capillaries, in which the inward and outward filtration of fluid depends on continually occurring variations in the capillary blood pressure produced by the vasomotion of the sphincters. Toward the venous end the variability of pressure shifts to favor inward filtration or ab-

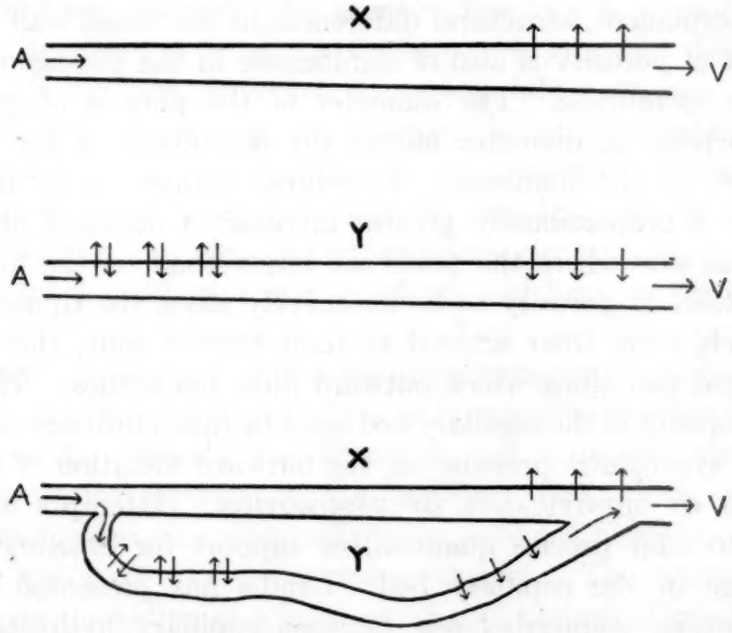

Fig. 1. Presenting three concepts regarding fluid movement in capillary bed.

sorption. The post-capillaries widen as they join the distal portions of the thoroughfare channels at an acute angle in such a manner that the blood in them is drawn into the channel analogous to the way that a vacuum jet operates.

Variations from the third sketch, *X* and *Y*, do occur, e.g., the precapillaries frequently lead off directly from terminal arterioles and the post-capillaries may lead directly into typical venules. However, the functional feature is the same, i.e., the intermittency of action of the precapillary sphincters and the peculiar arrangement of the post-capillaries. Fluctuations of pressure in the post-capillaries may occur from the occasional development of a significant venous back-pressure. Such fluctuations may change the direction of movement across the capillary wall.

The gradient of permeability in the thoroughfare channel seems to represent a gradation in the limiting dimensions of the pores along the wall of the vessel. This is indicated by introducing dyes of graded particle size into the circulation and noting their escape along the channel. The results have warranted the

conclusion that there is a gradual increase in the relative number of the larger pores toward the end where the blood is most venous.

The experiments of experimentally re-routing the venous blood indicate that the size and number of pores is a variable feature (38).

The direction of the gradient can be upset experimentally. By compression of appropriate channels with a microneedle, the flow through the capillary bed was reversed. After a short period of time a new gradient was established, an increasing porosity now becoming evident toward the original arterial side of the capillary bed. Thus, when blood from the venous vessels was re-routed back through the thoroughfare channel, a marked increase in the porosity of the vessel wall occurred, colloidal dyes such as T-1824, to which only the venous end is normally permeable, now passing out along its entire length. The gradient, therefore, appears to be a result of some factor in venous blood and not due to permanent, structural differences in the vessel wall.

A gradient of porosity is also of significance in the passage of water through the capillary membrane. The diameter of the pore is of prime importance since any increase in diameter affects the magnitude of the filtration to the second power of the diameter. Therefore, changes in hydrostatic pressure should cause a proportionally greater increase in outward filtration of water on the venous side where the pores are larger than on the arterial side. We find the gradient of porosity to be exclusively along the thoroughfare channels through which, from their arterial to their venous ends, the flow of blood is relatively rapid and along which outward filtration occurs. The existence of a gradient of porosity in the capillary bed must be taken into account in considering the effect of hydrostatic pressure on the outward filtration of fluid.

SUMMATION OF SIGNIFICANCE OF VASOMOTION. Attempts in the past have been made to offer precise quantitative support for Starling's hypothesis of fluid exchange in the capillary bed. Landis has presented calculations indicating a precise counterbalance between capillary hydrostatic and colloid osmotic pressure, the loss of fluid on the arterial side being compensated for by an equivalent intake of fluid on the venous side.

In the present review, the basic concept of Starling's hypothesis remains unchallenged. However, the balance of fluid exchange is considered to be maintained not so much by a direct interplay of hydrostatic versus colloidal pressures as by delicate vasomotor adjustments which intermittently increase or decrease the surface area over which an effective hydrostatic pressure produces outward filtration.

Inward filtration is accomplished primarily by osmotic uptake. The effectiveness of this mechanism, however, is directly dependent upon and is reinforced by the action of hemodynamic forces maintaining an adequate venous outflow from the capillary bed. Keys and co-workers (39) and Ralli and her co-workers (40) found that edema can occur with no concomitant reduction of the plasma proteins in the blood. Such data indicating the relative ineffectiveness of colloid osmotic pressure for preventing fluid loss from the capillary circulation emphasize the importance of factors other than the interplay of hydrostatic

vs. osmotic pressure in maintaining normal fluid exchange between the blood and the tissues. Their findings may be explained either by the assumption that the permeability of the capillary wall is so affected as to cause the loss of whole plasma, or that the loss of non-protein fluid in one region, e.g., the abdominal cavity or dependent parts of the extremities, is counterbalanced by an uptake of similar fluid from another region, e.g., skeletal muscle.

NERVOUS CONTROL OF CAPILLARY PERMEABILITY. It was claimed by Engel (41) that the sympathetic system induces an increase in capillary permeability, a view opposite to a previous one of Gellhorn (42) who presented evidence that sympathectomy induces an increase. Engel perfused the vessels of the knee-joint of a cat with fuchsin S in Ringer's solution and found that, although sympathectomy induced marked vasodilatation, the concentration of the dye in the knee-joint fluid was lowered. He ascribed this to a decreased permeability resulting from the absence of sympathetic nerve control. Danielli (cf. 9) discounted Engel's findings as indicating permeability changes and ascribed them to a lowered capillary pressure resulting from vasodilatation. Engel's results might more logically be explained by the opening of cross anastomoses (AVA) between the larger vessels so that the capillary bed in the particular region he was observing was bypassed by the active circulation.

Most authors favor Gellhorn's original thesis and have concluded that sympathectomy produces an increase in loss of fluid from the capillaries. This would be expected on the basis of the resulting hyperemia and increased blood flow. However, this does not necessarily signify any specific effect on endothelial permeability since the resulting vasodilatation and hyperemia should cause an increase in hydrostatic pressure in the capillary bed. This, together with suspension of vasomotion, should greatly favor outward filtration.

EFFECT OF AGENTS ON CAPILLARY PERMEABILITY. The literature contains many references to the action of a large number of biological agents in affecting the permeability of the capillary wall. A list taken from the more recent literature is presented in the accompanying table. Only those investigations are listed which deal with findings purporting to be directly related to the capillaries.

A commonly used technique (43) is that of observing the loss of dye from the blood into the skin during a local inflammatory reaction and noting the effect of various locally introduced agents in altering the amount of dye escape. Such studies shed little or no light on the mechanism of capillary permeability. Agents, such as desoxycorticosterone acetate, estrogens, and cortical extracts, which have been found to reduce the accumulation of trypan blue in an inflamed area (see table) do not necessarily produce their effect by acting on the capillary wall proper. For example, Rigdon (55) who used this method extensively has questioned the significance of the dye accumulation since he found that the stainability of the affected tissue is involved. The inflammatory reaction is a complex one, involving such diverse effects as vascular dilatation, endothelial damage, changes in the connective tissue matrix, blockage of lymphatic flow, stainability of the tissue, etc. The value of such data in clarifying capillary permeability phenomena is open to question.

TABLE 1

Agents affecting capillary permeability

AGENT	AUTHOR	EFFECT	CRITERION
Adrenal cortical secretions	Swingle et al. (43)	Maintenance of capillary tone	Adrenalectomy: increased permeability and capillary stasis
Adrenal cortical secretions	Cope et al. (44)	Decreased capillary permeability	Adrenalectomy: increased blood protein in lymph
Adrenal cortical extract DCA	Menkin (45)	Decreased capillary permeability	Leukotaxin effect of dye accumulation in skin was decreased
Adrenal cortical steroids Cortico sterone	Freed and Lindner (46)	Decreased capillary permeability	Same as above
Adrenal cortical extract	Shleser and Freed (47)	Decreased capillary permeability	Peptone effect of dye accumulation in skin was decreased
Adrenal cortical extract 11-desoxycorticosterone	Hyman and Chambers (48)	Reduced edema formation	Decreased weight of perfused hind-limbs of frog
DCA Cortin	Graham (49)	Decreased capillary permeability	Dye leakage by carbon arc irradiation of skin decreased
DCA	Fine and Fischmann (50)	No effect on capillary permeability	Rate of appearance of dye in tissues, disappearance of wheals; effect on albuminuria
DCA	Swingle and Remington (51)	Decreased capillary permeability	Increased retention of transfused serum in adrenalectomized dogs
Estrogens	Hechter et al. (52)	Increased capillary permeability in uterus and vagina	Increased concentration of dye in tissues
Alpha estradiol benzoate	Rigdon and Chrisman (53)	No effect	Xylol effect of local accumulation of dye in skin unchanged
Vitamin D	Silver et al. (54)	Decreased permeability with high doses	Decrease in disappearance rate of T-1824 from blood

TABLE 1—*Concluded*

AGENT	AUTHOR	EFFECT	CRITERION
Tissue extract	Rigdon (55)	Increased capillary permeability	Local injection induces accumulation of dye in skin
Leukotaxin	Menkin (56)	Increased capillary permeability	Local injection induces accumulation of dye in skin
Spreading-factor	Duran-Reynals (57)	Increased capillary permeability	Local injection induces accumulation of dye in skin
Histamine	Roche e Silva and Dragstedt (58)	Increased capillary permeability	Local injection induces accumulation of dye in skin
Histamine	McCarrell and Drinker (59)	Increased capillary permeability	Increased blood protein content of lymph
Histamine	Stead and Warren (60)	Increased capillary permeability	Decreased protein in venous blood
Histamine	Hechter (61)	Increased capillary permeability	Local dye accumulation unaffected by adrenalectomy
Acetylcholine	Gordon-Königes and Ottó (34)	Increased capillary permeability	Increase of lymph in lacteal of villus following dilatation of arterioles and increased capillary pressure

Other studies have used the appearance in the tissue of agents, the concentration of which can be measured, as an indication of permeability changes. Lange (62) measured skin fluorescence by a photometer after intravenous injection of sodium fluorescin. He found a decreased rate of dye accumulation in the skin under conditions such as myxedema. This dye is freely diffusible through the normal capillary wall. Evidence of increased fluorescence in the skin under experimental or pathological conditions may merely indicate an increased blood flow through the tissue with no actual increase in the permeability of the capillary wall to the dye.

A similar criticism can be made of experiments on the permeability of radioactive ions. Experiments with radioactive proteins (Fine and Seligman, 63; Cope and Moore, 64) should be of greater significance since proteins are normally retained by the capillary wall. The experiments of Fine and Seligman, which were performed on dogs in irreversible shock, indicated no outward leakage of

the radioactive proteins. On the other hand, Cope and Moore found an increased loss of radioactive colloids into the lymph following burns.

Of the various biological agents investigated, histamine and adrenal cortical extracts are of special interest. It has been claimed that histamine increases capillary permeability while the cortical extracts reduce it.

Histamine. The pioneer work of Lewis (65) and of Lewis and Grant (66) indicate that histamine-like substances produce a triple vascular response terminating in the marked exudation of protein-containing fluid from the capillaries into the tissues. Histamine, introduced into the skin, produces both arterial dilatation and damage to the capillary endothelium. In our experiments we found that histamine, introduced either locally into the mesentery with a micropipette or systemically by intravenous injection in concentrations not affecting arteriolar caliber, had no observable permeability effects (judged by dye exudation, stasis, etc.). Application with a micropipette in concentrations which produce dilatation of the arterioles also had no observable effects. Permeability changes were obtained only when concentrations were used high enough to produce actual endothelial damage, i.e., extravascular loss of normally retained Evans blue, sticking of carbon, swelling of individual endothelial cells and an abnormal distention of the capillary wall. To what extent histamine plays a rôle in normal permeability changes is still open to question.

Other experiments indicating a permeability-increasing effect of histamine have relied upon the loss of materials from the blood, e.g., proteins, which are normally retained within the capillary. This was detected by analysing the lymph fluid, the venous blood from affected regions or the fluid in various body cavities. Obviously, damage to the endothelium, such as occurs with excessive concentrations of histamine, will produce abnormal leakage not only of plasma but of blood cells.

In many cases the extravascular loss of protein can be explained equally well by vascular mechanisms other than increased capillary permeability. In this regard two techniques can be considered, that used by McCarrell and Drinker (cf. 59) and that by Stead and Warren (cf. 60). McCarrell and Drinker used changes in the constitution of the lymph of a particular region as an index of blood capillary permeability. They found that histamine shock produced an increase of protein in the lymph. Such an occurrence has a possible explanation from the findings of E. R. Clark (67). Clark noted that the application of pressure or increased temperature caused the passage of whole blood through the wall of venules directly into contiguous terminal lymphatics. This might be sufficient to explain the increase in protein content of the lymph without postulating an increase in capillary permeability *per se*. Stead and Warren determined differences in the contents of arterial and venous blood from affected areas. They noted that an injection of histamine (0.5 mgm.) into the brachial artery caused a decrease of the protein content of the venous blood of the same arm. This finding may be interpreted as an effect of the peculiar type of vascular reaction caused by histamine. Histamine produces arteriolar dilatation and

increases the blood flow through the capillary bed but it does not abolish vasomotion of the precapillary sphincters. This condition produces an excess of blood flow through the thoroughfare channels accentuating outward filtration all along their course. Normally, only a small amount of protein escapes from the venous portion of the channel. However, the increased hydrostatic pressure produced by histamine could be expected to lead to a greater leakage of protein in this region. Concomitantly, the true capillaries, which maintain their normal intermittent circulation because of the persistence of the precapillary sphincteric action, continue to perform their function of inward filtration undisturbed by the histamine. These two reactions would explain the lower protein content of the venous blood.

Adrenal cortical extracts. Much indirect evidence has accumulated to indicate that cortical extracts have a counteracting effect on the increase in capillary permeability produced by many noxious agents. Furthermore, in adrenal cortical insufficiency an increase in permeability is indicated by a pathological disturbance of fluid-exchange in favor of outward filtration and the production of a marked hemoconcentration (cf. 43). The question remains as to whether the observed effects are to be explained by the regulatory action of the cortical hormones on the distribution of blood to the tissues or whether these hormones serve to maintain the structural integrity of the capillary wall.

Hechter (cf. 61), by applying histamine through a stab in the skin of rats, observed no difference between normal and adrenalectomized rats in the extravascular loss of trypan blue into the region. Cope and co-workers (cf. 44) obtained an increased protein content in the cervical lymph of adrenalectomized dogs. They claimed this to indicate an increase in permeability of the blood capillaries to protein.

We have found, by direct observation of the capillary circulation, that bilaterally adrenalectomized rats (48 to 96 hrs. post-operative) show no visible alterations in the endothelial wall. Pathological changes of the capillary wall were detected only just prior to circulatory collapse preceding death. It is known that adrenalectomy induces the development of hemoconcentration. This might indicate increased capillary permeability. However, the excessive loss of fluid from the circulation can be explained equally well by an alteration of the hemodynamic relations of the capillary bed. We have found that adrenalectomized rats soon develop an atonic state of the peripheral arteries and arterioles and, subsequently, a cessation of vasomotion in the capillary bed. The resulting derangement of the capillary blood flow is one which favors outward filtration. The failing vasomotion and the consequent inadequate venous return, leading to increasing back pressure in the capillary bed, could account for loss to the tissues not only of water but also of proteins without considering that the integrity of the capillary wall has been impaired.

In the present article no attempt has been made to cover experimental findings dealing with the physiology of the more highly specialized components of the peripheral vascular apparatus, such as those dealing with temperature-regulating

mechanisms in the skin, special secretory barriers such as are obtained in the brain and complex structures observed in the liver and spleen, or in the bat's wing.

Although much has been written on permeability-changes in the capillary bed, most of the findings are largely inferential and only a few are based on direct observations of the vessels. Many of the findings are significant but, until the actual site of their action is ascertained, cannot be used for an understanding of the mechanisms involved.

The fact must be appreciated that the capillary bed has a highly specialized organization the several components of which maintain a delicately adjusted functional balance between reactions of central and local origin. The dominance of a central control tends to be countered by local autonomous control. Along with this ever-shifting balance of the hemodynamics are possibilities of physicochemical changes in the composition of the several different structures which constitute the wall of a capillary. The action of specific agents on one or more of the various components, structural and chemical, requires further intensive study.

Unless adequate precautions are taken and direct observational work is adhered to, it is generally impossible to differentiate between the effect of shifts in hemodynamic relationships, changes in functional permeability of the wall of capillaries, or actual disruptive leakages of the vessels. Most of the data in the literature deal with overall changes involving one or more of the above phenomena. Their physiological significance with respect to the control of capillary permeability will remain obscure until the site of action of the various agencies can be determined.

REFERENCES

(1) Chambers, R. The relation of extraneous coats to the organization and permeability of cellular membranes. C. S. H. Symp. Quant. Biol. **8**: 144, 1940.

(2) Keosian, J. Secretion in tissue cultures. III. Tonicity of fluid in chick mesonephric cysts. J. Cell. Comp. Physiol. **12**: 23, 1938.

(3) Hoeber, R. Ueber Resorption im Darm. Pflüger's Arch. **86**: 199, 1901.

(4) Krogh, A. The active and passive exchanges of inorganic ions through the surface of living cells and membranes in general. Croonian Lecture, Proc. Roy. Soc. Series B, **133**: 140, 1946.

(5) Florey, H. Capillary permeability. J. Physiol. (Proc.) **61**: 1, 1925.

(6) Field, M. E. and C. K. Drinker. The passage of visible particles through the walls of the capillaries. Am. J. Physiol. **116**: 597, 1936.

(7) Abell, R. G. The permeability of blood capillary sprouts and newly formed blood capillaries as compared to that of older capillaries. Am. J. Physiol. **147**: 237, 1946.

(8) Wilbrandt, W. Physiologie der Zell- und Kapillarpermeabilität. Helv. Med. Acta **13**: 143, 1946.

(9) Danielli, J. F. and A. Stock. The structure and permeability of blood capillaries. Biol. Rev. **19**: 81, 1944.

(10) Chambers, R. and B. W. Zweifach. Capillary endothelial cement in relation to permeability. J. Cell. and Comp. Physiol. **15**: 255, 1940.

(11) Landis, E. M. Capillary pressure and capillary permeability. Physiol. Rev. **14**: 404, 1934.

(12) BRINKMANN, R. AND A. SZENT-GYÖRGYI. Studien über die physikalisch-chemischen Grundlagen der vitalen Permeabilität. I. Die Wirkung kapillaraktiver Stoffe auf die Permeabilität von Kollodium-Membranen. Pflüger's Arch. **245:** 22, 1941.

(13) DANIELLI, J. F. Capillary permeability and edema in the perfused frog. J. Physiol. **98:** 109, 1940.

(14) KROGH, A. AND G. A. HARROP. On the substance responsible for capillary tonus. J. Physiol. **54:** Proc. cxxv, 1921.

(15) DRINKER, C. K. The permeability and diameter of the capillaries in the web of the brown frog when perfused with solutions containing pituitary extract and horse serum. J. Physiol. **62:** 249, 1927.

(16) HEIMBURGER, H. Beiträge zur Physiologie der menschlichen Kapillaren. V. Farbenversuche am Kapillarendothel und die Lymphräume des Papillarkörpergewebes. Ztschr. f. d. ges. exper. Med. **55:** 17, 1927.

(17) BENNINGHOFF, A. Ueber die Formenreihe der glatten Muskulatur und die Bedeutung der Rouget'schen Zellen der Kapillaren. Ztschr. f. Zellforsch. u. mikr. Anat. **4:** 125, 1926.

(18) MIDSUMO, R. Beiträge zur Morphologie und Physiologie der terminalen Blutbahn. Beitr. z. path. Anat. u. allg. Path. **84:** 183, 1930.

(19) MICHELS, N. A. The structure of the capillaries and the unmyogenic character of Rouget cells (pericytes) in the omentum of rabbits and in the web of living frogs. Anat. Rec. **65:** 99, 1936.

(20a) VOLTERRA, M. Einige neue Befunde über die Struktur der Kapillaren und ihre Beziehungen zur sogenannten Kontraktilität derselben. Zentralbl. f. inn. Med. **46:** 876, 1925; Ricerche sul Sistema redicoloistiocitario. Lo Sperimentale **81:** 319, 1927; Ulteriori studi sulla morfologia e sulla funzione dei capillari sanguigni. Arch. ital. di Anat. e di Embriol. **33:** 844, 1934.

(20b) VOLTERRA, M. Ueber die Pathogenese der Nierenblutungen und ihre anatomischen Ursachen. Zentralbl. f. inn. Med. **36:** 857, 1926.

(21) GOREV, N. N. AND A. J. SMIRNOVA-ZAMKOVA. Observations on the pathogenesis of hypertension. Am. Rev. Sov. Med. **3:** 28, 1945.

(22) DURAN-REYNALS, F. Tissue permeability and spreading factor in infection. Bact. Rev. **6:** 197, 1942.

(23) MCCLEAN, D. A factor in culture filtrates of certain pathogenic bacteria which increases permeability of the tissues. J. Path. and Bact. **42:** 477, 1936.

(24) BARON, H. AND R. CHAMBERS. A micromanipulative study on the migration of blood cells in frog capillaries. Am. J. Physiol. **114:** 700, 1936.

(25) CHAMBERS, E. L. Unpublished data.

(26) CHAMBERS, R. AND B. W. ZWEIFACH. Topography and function of the mesenteric capillary circulation. Am. J. Anat. **75:** 173, 1944.

(27) LANDIS, E. M. Micro-injection studies of capillary permeability. II. The relation between capillary pressure and the rate at which fluid passes through the walls of single capillaries. Am. J. Physiol. **82:** 217, 1927.

(28) MCMASTER, P. D. Intermittent take-up of fluid from the cutaneous tissue. J. Exper. Med. **73:** 67, 1941.

(29) STARLING, E. H. The fluids of the body. London, A. Constable & Co., 1909.

(30) LANDIS, E. M. Capillary pressure and hyperemia in muscle and skin of frog. Am. J. Physiol. **98:** 704, 1931.

(31) LANDIS, E. M. The capillary blood pressure in mammalian mesentery as determined by the micro-injection method. Am. J. Physiol. **93:** 353, 1930.

(32) LANDIS, E. M. Micro-injection studies of capillary blood pressure in human skin. Heart **15:** 209, 1930.

(33) LANDIS, E. M. The capillary pressure in frog mesentery as determined by micro-injection methods. Am. J. Physiol. **75:** 548, 1926.

(34) GORDON-KÖNIGES, H. AND M. OTTO. Studies on the filtration mechanism of the intestinal lymph and on the action of acetylcholine on it and on the circulation of the intestinal villi. Quart. J. Exper. Physiol. **26**: 319, 1937.

(35) ROUS, P., H. P. GILDING AND F. SMITH. Gradient of vascular permeability. J. Exper. Med. **51**: 807, 1930.

(36) LANDIS, E. M. Capillary permeability and the factors affecting the composition of capillary filtrate. Annals N. Y. Acad. Sc. **46**: 713, 1946.

(37a) SMITH, F. AND M. DICK. Influence of plasma colloids on gradient of permeability. J. Exper. Med. **56**: 371, 1932.

(37b) MCMASTER, P. D., S. HUDACK AND P. ROUS. The relation of hydrostatic pressure to the gradient of capillary permeability. J. Exper Med., **55**: 203, 1932.

(38) ZWEIFACH, B. W. The structural basis of permeability and other functions of blood capillaries. C. S. H. Symp. Quant. Biol. **8**: 216, 1940.

(39) KEYS, A., H. L. TAYLOR, O. MICHELSON AND A. HENSCHEL. Famine edema and the mechanism of its formation. Science **103**: 669, 1946.

(40) RALLI, E. P., J. S. ROBSON, D. CLARKE AND C. L. HOAGLAND. Factors influencing ascites in patients with cirrhosis of the liver. J. Clin. Investigations **24**: 316, 1945.

(41) ENGEL, D. The influence of the sympathetic nervous system on capillary permeability. J. Physiol. **99**: 161, 1940.

(42) GELLHORN, E. Das Permeabilitätsproblem. Berlin, Springer, 1929.

(43) SWINGLE, W. W., W. M. PARKINS AND A. R. TAYLOR. A study of the circulatory failure of adrenal insufficiency and analogous shock-like conditions. Am. J. Physiol. **123**: 659, 1938.

(44) COPE, O., A. G. BRENIZER, JR. AND H. POLDERMAN. Capillary permeability and adrenal cortex studies of cervical lymph in the adrenalectomized dog. Am. J. Physiol. **137**: 69, 1942.

(45) MENKIN, V. Effect of adrenal cortex extract on capillary permeability. Am. J. Physiol. **129**: 691, 1940.

(46) FREED, S. C. AND E. LINDNER. The effect of steroids of the adrenal cortex and ovary on capillary permeability. Am. J. Physiol. **134**: 258, 1941.

(47) SHLESER, I. H. AND S. C. FREED. The effect of peptone on capillary permeability and its neutralization by adrenal cortical extract. Am. J. Physiol. **137**: 426, 1942.

(48) HYMAN, C. AND R. CHAMBERS. Effect of adrenal cortical compounds on edema formation of frog's hind limbs. Endocrinology **32**: 310, 1943.

(49) GRAHAM, J. S. Effect of carbon arc irradiation and adrenal cortical preparations on capillary permeability. Proc. Soc. Exper. Biol. and Med. **54**: 101, 1943.

(50) FINE, J. AND J. FISCHMANN. A study of the effect of desoxycorticosterone acetate on capillary permeability. Proc. Soc. Exper. Biol. and Med. **49**: 98, 1942.

(51) SWINGLE, W. W. AND J. W. REMINGTON. The effect of DCA and of blood serum transfusions upon the circulation of the adrenalectomized dog. Am. J. Physiol. **134**: 503, 1941.

(52) HECHTER, O., L. KROHN AND J. HARRIS. Effects of estrogen and other steroids on capillary permeability. Endocrinology **30**: 598, 1942.

(53) RIGDON, R. H. AND R. B. CHRISMAN, JR. Effect of alpha estradiol benzoate on local areas of inflammation in the skin of the rabbit. Endocrinology **28**: 758, 1941.

(54) SILVER, A., I. E. STECH AND C. I. REED. A study of the effects of vitamin D on capillary permeability by use of the dye T-1824. J. Lab. Clin. Med. **29**: 48, 1944.

(55) RIGDON, R. H. Demonstration of a capillary permeability factor in tissue extracts from normal rabbits. Arch. Surg. **41**: 96, 1940.

(56) MENKIN, V. Studies in inflammation. J. Exper. Med. **64**: 485, 1936.

(57) DURAN-REYNALS, F. A general permeability increasing effect of a factor from mammalian testicles on blood capillaries. J. Biol. Med. **11**: 601, 1939.

(58) Roche e Silva, M. and C. A. Dragstedt. Nature of the capillary permeability factor present in extracts of normal tissues. Proc. Soc. Exper. Biol. and Med. **46:** 303, 1941.

(59) McCarrell, J. D. and C. K. Drinker. Cervical lymph production during histamine shock in the dog. Am. J. Physiol. **133:** 64, 1941.

(60) Stead, E. A., Jr. and J. V. Warren. The effect of the injection of histamine into the brachial artery on the permeability of the capillaries of the forearm and hand. J. Clin. Investigation **23:** 279, 1944.

(61) Hechter, O. Effect of histamine upon capillary permeability in the skin and muscle of normal and adrenalectomized rats. Endocrinology **32:** 135, 1943.

(62) Lange, K. and S. E. Krewer. The dermo-fluorometer. J. Lab. Clin. Med. **28:** 1746, 1943.

(63) Fine, J. and A. M. Seligman. A study of the problem of the lost plasma in hemorrhage, tourniquet and burn shock by the use of radioactive iodo-plasma protein. J. Clin. Investigation **23:** 720, 1944.

(64) Cope, O. and F. D. Moore. A study of capillary permeability in experimental burns and burn shock using radioactive dyes in blood and lymph. J. Clin. Investigation **23:** 241, 1944.

(65) Lewis, T. Blood vessels of the human skin and their responses. London, Shaw and Sons, Ltd., 1927.

(66) Lewis, T. and R. T. Grant. Vascular reactions of the skin to injury. II. Liberation of a histamine-like substance in injured skin. Heart **11:** 209, 1921.

(67) Clark, E. R. Intercellular substance in relation to tissue growth. Annals N. Y. Acad. Sc. **46:** 733, 1946.

SOME PHYSIOLOGICAL EFFECTS OF CURARE AND THEIR APPLICATION TO CLINICAL MEDICINE

A. R. McINTYRE

Department of Physiology and Pharmacology, University of Nebraska, College of Medicine, Omaha

The revival of interest in curare and the important place it has lately won in medicine and surgery is likely to obscure the fact that the study of South American arrow poisons was among the earliest of toxicological investigations. It was largely out of these studies that modern physiology took root and grew. The complex story of the history, botany and chemistry of the curares is remarkable and has interested physicians for centuries. Unfortunately it will not be possible to enter into a discussion of these matters here; they have, however, recently been described in detail elsewhere (1).

It is the purpose of this review to present the gist of to-day's knowledge of the physiology and pharmacology of curare, to outline some of the implications of this knowledge regarding neuromyic transmission and to describe the practical application of this drug to clinical usage. It should first be pointed out, however, that a large variety of South American arrow poisons are indiscriminately referred to as "curares," many of which differ markedly in composition. Comparatively little experimental work has been performed with the pure alkaloids obtainable from them, and of these, only two have been widely used. These are curarine, a potent amorphous alkaloid of unknown chemical structure isolated by Boehm (2), and d-tubocurarine, a bisbenzylisoquinoline compound first obtained in a pure state from tube-curare by King (3), who worked out its chemical structure. Subsequently d-tubocurarine was obtained by Wintersteiner and Dutcher (4), of the Squibb Institute, from *Chondrondendron tomentosum*, a member of the Menisperm or moonseed family, and this alkaloid, accordingly, has been rendered readily available for experimental work. Almost all the clinical work with curare during the last seven years has been performed with Intocostrin, a partially purified and biologically standardized solution of certain varieties of crude curare; the precursor of this preparation was first prepared in the author's laboratory.

The classical effect of curare. In spite of the enormous amount of experimentation performed during the ninety years which have elapsed since Claude Bernard (5) wrote, "Le Curare, qui anéanit l'action nerveuse sur les muscles, conserve au contraire plus longtemps la contractilité musculaire. Preuve que ce sont là deux actes bien distincts," the exact mode of action of curare remains a matter of dispute. The locus of action is generally agreed upon and is thought to reside in the somewhat ill-defined region where nervous tissue ends and muscle tissue begins. The question as to curare's mode of action will, of course, remain a bone of contention until there is complete agreement among physiologists and pharmacologists concerning the *modus operandi* of neuro-muscular transmission. This is not the place to plead the cause of either of the two chief theories of indirect

excitation of muscle, but rather to examine how well the established facts concerning curarization fit the theories. Quite understandably, Claude Bernard's localization of the point of action of curare at the neuromyic junction focussed attention of investigators on this region and it was Bernard's pupil, Kühne (6), who scrutinized nerve endings minutely in an effort to find answers to the yet unanswered questions concerning curare's exact pharmacological action. Out of Kühne's work came a knowledge of the anatomy of the motor nerves and their peculiar endings now known as "motor end-plates." It was found that in the lizard, during life, the motor end-plates were thin, translucent and almost invisible but, after death or after the application of strong curare solutions, the plates became more opaque and clearly visible. Since the days of Kühne a large number of investigators have examined the motor endings in many species. There is much variation in these structures and in some varieties of frogs there are no large terminal plates, the motor nerves "run into the muscle" with a few small knob-like structures scattered along their terminal twigs, thus Langley (7) maintained that in the frog there was no evidence of the presence of any tissue which could not be identified with certainty as either nerve or muscle. Most, but not all, investigators (8) are agreed that high concentrations of curare will cause changes in the appearance of motor nerve terminations. Recently Carey (9) has described many interesting effects on the motor end-plates, not only as the result of the application of strong curare solutions but also following electrocution and poisoning with carbon monoxide, tetraethyl lead, and prostigmine. Some experimenters have reported that, with lower concentrations of curare, morphological changes in the end-plates of curarized muscle are not found, and King and Willard (10) using d-tubocurarine in adequate concentrations to produce curarization, could find no change in the appearance of the motor end-plates; it was not possible for them to differentiate, from their histological appearance, which specimens were curarized and which were not. Accordingly, while it is undoubtedly true that curare and other poisons are capable of affecting visible changes in the end-plates, these changes are not necessarily specific for curarization. Furthermore, these changes have not, as yet, been demonstrated to be reversible.

Langley's conclusion from experiments in the first few years of the present century are hardly reconcilable with the theory that curarization is brought about by an effect on the motor end-plates. It will be recalled that he found that, in denervated muscle, nicotine will cause contractions, and he showed that these nicotine-provoked contractions are preventable by curare. As mentioned above, Langley found that in the frog there is no difficulty in deciding which tissues were nerve and which tissues were muscle. Hence, in a denervated preparation, the effect of curare was evidently on muscle-tissue itself. Langley's extensive experiments with curare and other drugs led eventually to his "receptor" theory of drug action and his work was an important factor in the subsequent acceptance, by many, of the chemical transmitter theory. However, the concepts of Elliot, Dixon, and Loewi were needed before the chemical transmission theory of neuromyic transmission was extended to striated muscle by

Dale and his co-workers. In the meantime the work of the Lapicques emphasized the electrical aspects of excitability. An electrical transmission mechanism is implicit in their theories and their work strongly influenced physiological thought concerning neuro-muscular physiology for many years.

Assuming for the moment that neuromyic transmission is accomplished simply by the shift in electrical potential accompanying the nerve impulse—curarization could then be due either to a suppression, partial or complete, of nerve impulses at their terminals, or to a change in the muscle brought about by curare which results in a raised electrical threshold. Obviously there is considerable difficulty in determining whether or not the ultimate terminals of motor-nerve conduct impulses. Kühne's (11) well known attempts to solve this question by the use of the branching motor-nerves in the frog's gracilis muscle which enabled him, by means of a longitudinal cut, to divide the muscle in such a manner that one portion could be soaked in "strongest curare solution" while the other portion remained free of curare, convinced him that the motor nerves, including their endings, were entirely unaffected. Unpublished investigations made in the author's laboratory have shown that d-tubocurarine in concentrations up to a hundred times greater than those necessary for curarization, are powerless to affect nerve-conduction in the frog sciatic, this was true after 18 hours' exposure of the nerve to the poison—but it has not yet been definitely shown whether or not curare in some way prevents the spread of the impulse from the axon over the terminal surface of the nerve. Kuffler (12) has reported experiments that show, following the application of curare to the myo-neural junction, a progressive decrease in the end-plate spike-potential. He points out that unless the leads are placed in close proximity to the end-plate region it is not possible to detect this diminution. As Kuffler himself remarks, "it would be of interest to know the threshold e.p.p. required for a propagated impulse." It is important to observe that the end-plate potential is depressed, not abolished, by curare.

The second possibility, that curare raises the electrical threshold of muscle, brings us at once to the much disputed theory of Lapicque. It is unnecessary to discuss this theory at length, nor is it necessary to present the views of Lapicque's opponents. This dispute has been reviewed in this journal by Davies and Forbes (13). It will suffice to say that neither the doctrine of isochronism for normal nerve and muscle nor the belief that heterochronism exists in curarization is acknowledged universally. This should not be allowed to detract from the usefulness of Lapicque's concept of chronaxie, nor should it be forgotten that Lapicque's theory of curarization (14) has been of great value in stimulating investigations in nerve and muscle physiology during most of the first half of this century. However, in view of the divergent views regarding the strength-duration curves of muscles and their motor nerves, and the many disagreements regarding the validity of the various techniques employed, (for details of which see the review by Davis and Forbes referred to above) it is obvious that any theory of curarization based on changes in *electrical* threshold of muscle is likely to meet with much justifiable criticism.

According to proponents of the chemical theory of neuromyic transmission,

curarization results either from failure of production of transmitter or from failure of the transmitter to accomplish its normal effect upon muscle. There is no evidence at all for the former but considerable evidence for the latter view. This evidence is as follows: Dale and co-workers have shown that muscles perfused with physiological solutions, when stimulated by their motor nerves, either before or during curarization, liberate an acetylcholine-like substance into the perfusate (15). In the writer's laboratory we have succeeded in showing that, in the eserinized dog, the venous blood from muscles, stimulated by their motor nerves, both before and during curarization, contains a substance capable of stimulating rabbit gut *in vitro* and arresting the perfused frog heart. These effects on the rabbit-gut and frog-heart by the venous blood are prevented by atropine. The substance contained in venous blood from stimulated muscles was found to differ in its effects upon the test tissues from those caused by excess potassium; thus in control experiments the effects of eserinized venous muscle blood, to which potassium was added, were compared with the effects obtained with those of eserinized blood obtained from stimulated muscles, the effects of the latter were more readily abolished by atropine than those of the former (16). Further evidence that curarization cannot be explained on the basis of failure in the production of the transmitter is provided from experiments in which acetylcholine is injected directly into an artery. As is well known, muscle-contractions closely simulating those obtained by stimulation of the motor nerve may thus be obtained. Following the injection of curare subsequent injections of acetylcholine are less effective (17, 18, 19), an observation obviously indicative that the action of curare is one of suppression of the effect of the transmitter rather than its failure of production. In denervated muscle the intra-arterial injection of acetylcholine readily arouses muscle-contractions and larger amounts of acetylcholine when so injected may cause muscle contracture accompanied by electrical silence. Curare prevents the action of acetylcholine in denervated muscles, and d-tubocurarine, when injected rapidly in sufficient concentration, is capable of first increasing the fibrillation and may then cause contractures of denervated muscle; during the contractures the spontaneous fibrillations are suppressed or abolished, and during this time muscles will not respond to acetylcholine (20). These findings are supporting evidence for Langley's belief that the site of action of curare is the muscle "effector substance" (see above), and prove beyond question that curare exerts an action on muscles in the absence of their nerves.

Pharmacological antagonists to curare. Eserine and neostigmine and related substances containing a methyl-carbamic ester group abolish the action of curare. These substances have been extensively examined by Briscoe (21) and others (22). They are known to inhibit the activity of serum acetylcholine esterase and this fact is frequently used to explain their pharmacological actions. This explanation is, unfortunately, not entirely adequate as is indicated by the following facts. Wilson and Wright (23) have shown that acetylcholine injected intra-arterially into curarized muscles has but little effect; however, the combined effect of the injection of acetylcholine and prostigmine is greater than either in-

jected alone. These authors have also shown that potassium greatly accelerates the decurarization effect of prostigmine. We find that the potency of Intocostrin (which inhibits acetylcholine esterase) is not measurably inferior to that exhibited by the same quantity of d-tubocurarine contained in it, although d-tubocurarine itself has little or no inhibitory effect on the above enzyme (24).

The antagonism of calcium to curare is of remarkable interest and it has been shown by Eccles and co-workers (25), Feng (26), and others (27), that the restoration of indirect excitability following the local application of calcium to the curarized nerve-muscle junction is accompanied by an increase in the spike potential of the end-plate region. However, it has also been shown that the exposure of a nerve muscle preparation to a solution *low* in calcium increases enormously the sensitivity of the muscle to the intra-arterial injection of acetylcholine (28). On the other hand, it is said that in the intact animal, the administration of parathyroid hormone raises the tolerance for curare (29). Dihydrotachysterol also is alleged to have the same effect (30). On the basis of present knowledge these somewhat paradoxical observations are difficult to harmonize. However, unreported experiments from this laboratory indicate that calcium-lack decreases the resting potential of motor nerve and that this decrease in resting potential is closely associated with the increase in sensitivity of the muscle to acetylcholine, as observed by Kuffler (1944). Furthermore, this increase in sensitivity to acetylcholine is the probable cause of the repetitive discharges following a single nerve volley such as possibly occurs in some forms of tetany. The beneficial effect of curare in tetany may be, in part, due to the raised threshold of muscle to acetylcholine which reduces, or perhaps abolishes, repetitive muscle responses. However, according to Kuffler (1944), curarine does not prevent the twitches of frog muscle obtained *in vitro* upon exposure of the muscle to low calcium; on the contrary we find in preliminary and unreported experiments that the addition of d-tubocurarine to the low calcium solution decreases the spontaneous activity of frog muscle immersed in it.[1]

The antagonistic effect of epinephrine upon curarization has been attributed to the effect of this substance on tissue permeability (31). However, according to Mies (32), the effect is indirect and is in part due to the action of epinephrine on the blood vessels in close proximity to the nerve-muscle junction. Certainly augmentation of blood-flow in muscle is likely to shorten the duration of action of curare. Guanidine, one of the most powerful decurarizing agents (33), appears, like calcium, to raise the spike-potential in the vicinity of the motor end-plate when single stimuli are applied to the nerve.

The reviewer has advanced the hypothesis that many of the effects of curare, acetylcholine, and other substances possessing pharmacological actions at the region of the junction between muscles and their motor nerves (34), can be harmoniously composed by the assumption that the function of the motor nerve is, in reality, inhibitory to the muscle fibres which otherwise undergo spontaneous

[1] Subsequently reported, see Wendt, Bernard F., and A. R. McIntyre. Proc. Am. Physiol. Soc. **6**: 224, 1947.

and rhythmic contractions. This inhibition is attributed to the positive (resting) charge on the axon terminal surface which, by its presence, is supposed to favor acetylcholine esterase activity in the region and effectively prevent the accumulation of significant concentrations of acetylcholine. When the surface of the nerve termination becomes more negative as a result of the arrival of a motor impulse, or as a consequence of injury, a reversal of the activity of the enzyme system is now hypothecated, and choline is thus acetylated. The resulting acetylcholine in turn effects the muscle "receptor surface" which is depolarized and the muscle contracts. This hypothesis accounts for the interrelationship between calcium ion concentration and acetylcholine sensitivity, because, according to this concept, a reduction of the resting potential of the motor end-plate would cause an increase in the concentration of acetylcholine, thereby increasing the effectiveness of any acetylcholine applied. We find that increasing the concentration of calcium ions in the fluids surrounding nerve, increases the positive resting potential at its surface. According to the above hypothesis this would tend to keep the activity of acetycholine esterase high and consequently the concentration of acetylcholine at a minimum, and may, if the concentration of calcium be sufficient, result in a block of neuro-muscular transmission. According to this hypothesis the spontaneous fibrillation of denervation should be interrupted by curare, but Solandt and Magladery (35) report that neither anesthetics such as ether and the barbiturates nor atropine, curare and eserine prevent the fibrillation of denervation; we find that d-tubocurarine injected intra-arterially into denervated fibrillating muscles will, when injected sufficiently rapidly, interrupt spontaneous fibrillation as electrically recorded by standard cathode ray techniques. (See ref. 20.) Feinstein et al. (36) have shown that it is very important to control the temperature of muscles in such experiments, and some of the above discrepancies may possibly be accounted for on this basis. In our experiments the blood supply was interrupted only momentarily and significant temperature changes did not occur.

Effects of curare on the autonomic nervous system and central nervous system. Interest in the classical action of curare has resulted in other pharmacological aspects of curare being somewhat neglected, but these are important. For example, curarine applied to autonomic ganglia is capable of blocking synaptic transmission in these structures (37). With concentrations of d-tubocurarine adequate for curarization of striated muscle, in our experience, this effect occurs rather slowly (38). The interruption of transmission at autonomic ganglia appears to be the result, not of interference with the production of acetylcholine, but due to interruption of its effectiveness at the surface upon which it normally acts. This is strongly indicated by experiments performed by us in which a frog heart was perfused with the perfusate from a turtle heart. The concentration of ions in the solution used for perfusion was carefully adjusted so as to be compatible with both the (donor) turtle heart and the (recipient) frog heart, though, unavoidably, less so than solutions best suited to one or the other. When the turtle vagus was stimulated the arrest of the turtle heart was promptly followed by arrest of the (recipient) frog heart. By diverting the perfusate

from the (recipient) frog heart during curarization of the donor heart and then, during the stimulation of the turtle vagus, again allowing the perfusate to flow through the frog heart, it was sometimes possible to obtain arrest of the recipient heart without arrest of the donor heart. This strongly indicates that although stimulation of the vagus produced *vagus-stoffe* in adequate quantities to arrest the recipient heart it was unable to arrest the curarized turtle donor heart. It should be pointed out that generally both the donor and recipient hearts were arrested upon stimulation of the turtle vagus. Arrest of the recipient heart *only* was seen in experiments in which the application of curare had been prolonged. These and other experiments with curare lead us to believe that, in general, cholinergic structures are more easily affected than adrenergic structures. At first possibly both are stimulated transiently, and later paralyzed, so that autonomic transmission from pre- to post-ganglionic neurones gradually fails and may cease entirely after exposure for sufficiently long periods of time to adequate concentrations of the drug. The effects of curare on the secretions are many. The literature is full of contradictions and inconsistencies. This is because the effects of curare upon the glands are probably mediated by its effects upon the autonomic system and the results obtained depend upon the duration of the experiments and the amount of curare used. Another factor responsible for divergent reports is, no doubt, the differences in the varieties of curare used. The following facts seem to be fairly well established. As a result of curarization, the blood sugar rises. The urine output falls without a significant change in blood-pressure. We suggest, without complete evidence, that the diminished urine secretion is the result of an increase in output of posterior pituitary hormone—the urine during curarization frequently contains much sodium chloride. The tolerance to insulin is increased. Somewhat surprisingly, when the convulsions in febrile conditions are controlled by curarization, the body temperature remains elevated. A large number of observations concerning miscellaneous actions of curare reported by numerous investigators have been collected by the writer (39); space does not permit their quotation here.

The effects of curare on abnormal muscle-tone. The effects of curare on the hypertonicity of muscle in decerebrate animals was first investigated by Bremer (40), who showed that curare was capable of reducing the muscle-tonus in such animals. This was accomplished without the production of classical curarization, the animals being able to walk without noticeable impairment of their gait. Somewhat parallel observations were reported by Hartridge and West (41) in tetany in dogs. Such animals could be protected from tetanic convulsions but at the same time retain their ability to walk, apparently, normally. West (see ref. 31), reported that there was considerable variation in the effectiveness of tetany-control by different curares, some possessing what he termed a "lissive" action, whereas others, including d-tubocurarine, possessed little or no lissive action. This alkaloid, as above mentioned, is the active alkaloid contained in Intocostrin. Consequently it is somewhat surprising to find that Intocostrin and d-tubocurarine are today the preparations chiefly used clinically in the treatment of spasticities. Burman (42), who was the first to report on the

use of curariform drugs in the treatment of spastic patients in this country, made his initial investigations before Intocostrin was available, and the botanical source of the curare he used is not entirely clear. Later Harvey and Masland (43) reported unfavorably on the results of curare in spastic conditions. The writer has had the opportunity of seeing a number of spastic patients who appeared to be benefited by the use of Intocostrin, and others, notably some of Doctor Schlesinger's patients, who seemed undoubtedly improved by the intramuscular injection of d-tubocurarine suspended in oil and beeswax, the effects of which are said to persist for as long as three days (44).

These clinical observations confront the pharmacologist with a difficult problem because in normal dogs the classical peripheral effects of these preparations do not, in the reviewer's experience, persist sufficiently long to account for the duration of the clinical improvements reported. There is also difficulty in explaining Bremer's observation (l.c.) on decerebrate rigidity and those of Hartridge and West (l.c.) in parathyroid tetany in terms of the classical curare effect, because, as mentioned above, these experimenters did not obtain measurable interference with the animals' ability to walk. It would seem that in the event of the curare preventing a certain proportion of the motor-nerve impulses responsible for the increased muscle tonus from reaching the muscles there would be an impairment of muscle-function roughly proportional to the decrease in the hypertonicity. Should it be demonstrated that in spastic states, repetitive muscle responses follow single motor nerve impulses, which seems rather unlikely, the beneficial effects of curare in spasticities would be more readily understandable. During curarization, as is well known, a muscle will rapidly lose its ability to maintain a tetanic contraction and then respond by a single twitch to an indirect tetanic stimulus. This phenomenon, however, is hardly adequate to explain the relief believed to be afforded by curare in hypertonicity. The question is further complicated by the fact that West (l.c.) was unable to find a lissive action with d-tubocurarine. Admittedly knowledge of the numerous factors responsible for pathological hypertonicity is incomplete and accordingly a discussion of the mode of action of curare in these conditions is consequently speculative rather than factual. It seems possible that the effects of curare in spastic states may depend, at least in part, upon some mechanism other than its classical peripheral action. Accordingly attention is drawn to the observations of Denhoff and Bradley (45), who state that their spastic patients experienced subjective manifestations following injection with effective doses of curare. These subjective symptoms might be interpreted as suggestive evidence of a central nervous system action of the drug, and West (46) has quoted the words of one of his patients whose subjective reactions, while under curare therapy, clearly indicated marked disturbances of the sensorium. The pharmacological actions of curare on the brain and cord have been largely neglected but convulsions, following intra-aortic injections, have long been known to occur (47), and when curare gains access to the ventricles marked disturbances of the C.N.S. result (48). Both Harlow (49) and Girden (50) have investigated the effects of curare on conditioned reflexes in dogs and both report changes in the responses of cur-

arized animals which they attribute to central actions. Girden believes that the curarized dog suffers amnesia. The electrical activity of the frog's C.N.S. has been investigated by Pick and his associates (51), who find a depression of activity which may last several days in the curarized animal. We find that the immediate effect of rapid injections of d-tubocurarine into anesthetized dogs is a transient increase in amplitude of the electroencephalographic record promptly followed by a decreased electrical activity of the brain (52). It is possible to cause sudden and complete arrest of respiration and death by the rapid intracarotid injection of d-tubocurarine; respiration may cease before indirect excitability of the diaphragm is lost. It is desirable that the mechanisms responsible for the decrease in muscle hypertonicity observed clinically be investigated with the possible rôle of these actions of curare on the C.N.S. in mind. It is conceivable that the effect obtained in spastic patients involves actions of curare on reflex pathways, resulting in impairment of synaptic transmission, which in the abnormal C.N.S. may be sufficiently modified by curare to account, in part, for the clinical benefits claimed. The subject obviously requires further investigation.

The use of curare in shock-therapy and anesthesia. At the suggestion of the author, A. E. Bennett (53) began the use of a partially purified and carefully standardized solution of curare, prepared in this laboratory, on psychopathic patients undergoing metrazol shock-therapy. The incidence of trauma resulting from the convulsions was reduced to the vanishing point, without diminishing the ratio of successful to unsuccessful treatments. Subsequently with the development of electro-shock therapy the use of curare in shock-therapy has continued and is today used in perhaps a majority of institutions where this type of therapy is employed. It is probable that the dosage of curare for use with electro-shock therapy should be somewhat less than that formerly used in metrazol shock-therapy. The reason for this is that metrazol is antidotal to curare whereas electro-shock-therapy is not. Electroencephalographic records, obtained by us, indicate that both curare and electro-shock result in decreased electroactivity of brain, hence, when both are employed together, they may augment each other.

Griffith and Johnson (54) introduced the use of curare as an adjuvant in anesthesia and Griffith (55) has extensively investigated the usefulness of the drug in this field. Cullen (56) has also investigated curare in anesthesia and Gross and Cullen (57) have made the important observation that with ether the amount of curare necessary to obtain good muscle-relaxation is considerably less than with such anesthetics as cyclopropane. In the opinion of the author cyclopropane anesthesia with curare approaches the ideal in anesthesia for a wide variety of surgical procedures. The deep relaxation obtained with curare supplies the one deficiency of cyclopropane. In abdominal surgery another advantage of curare is that the gut is rendered "small" and inactive. Presumably this effect on the gut is caused by an effect upon synaptic conduction in the autonomic system because we find that the concentrations necessary to modify rabbit jejunum *in vitro* far exceed those likely to be present when the drug is used in man. While the chief value of curare in anesthesia undoubtedly resides in the muscle relaxa-

tion obtained due to the drug's classical peripheral action, there is disagreement as to what rôle, if any, is played by its central actions. There have been a number of papers which report the administration of very large doses of curare to patients, either by accident or by design, in which it appeared that anesthesia resulted, and in some instances major surgery has been successfully accomplished under curare alone (58). On the other hand, Dr. Scott M. Smith (59) allowed himself to be injected with d-tubocurarine to the point of complete curarization without the loss of consciousness. It is possible that the rate of administration of d-tubocurarine in Smith's experiment was insufficiently great to produce the effects reported by others. This important question requires further investigations. It is, however, not too easy to find competent observers who exhibit the devotion to science shown by Doctor Smith.

The use of curare for the control of convulsions in tetanus dates back to the early part of the 19th century and the literature on this topic has been previously reviewed by the author (60). There is no evidence at present that curare is capable of saving the patient's life although control of convulsions can be rather readily accomplished. One of the difficulties in the prolonged use of curare for the maintenance of deep curarization is that, in experimental animals at least, the animal gradually succumbs. The mechanism of death is obscure (61). The reviewer believes that death is related to the gradual interruption of function of the C.N.S. brought about by the prolonged action of curare. In spite of this the experimental use of curare in severe human tetanus is fully justified when adequate facilities for maintaining effective artificial respiration are available. Reliance on atmospheric pressure to fill the lungs, as for example, when an "iron lung" is used, may result in inadequate pulmonary ventilation due in part to bronchial spasm. Methods employing positive pressure should therefore be used.

Ransohoff (62) has taken advantage of curare to decrease the painful muscle spasm frequently found early in poliomyelitis, thereby rendering his patients more comfortable and facilitating the early application of physio-therapy. On the basis of present knowledge it should not be assumed that there is any *direct* beneficial effect exerted by curare.

The only known contraindications to the use of curare are during the acute paralytic stage of poliomyelitis and in *myasthenia gravis*, a disease in which sensitivity to curare is markedly increased. The many parallelisms between partial curarization and *myasthenia gravis* have been described by Bennett, McIntyre and Cash (63). The antidotal value of eserine and prostigmine bromide have been compared by the author (64) and the former was found to be superior; however, subsequent work has shown that prostigmine *methyl sulfate* is fully as effective as eserine.

It is unnecessary to enumerate all the clinical applications of curare which have been attempted; they include the treatment of such diverse conditions as persistent singultus, dysmenorrhea, epilepsy and as an aid in endoscopy. They have been discussed in some detail elsewhere (65). If the claim of the successful treatment of dysmenorrhea is substantiated it is obvious that the practical

pharmacological use of curare is not necessarily limited to its classical effect on skeletal muscle.

SUMMARY

1. The mechanism of curarization cannot be completely explained until the problem of neuro-muscular transmission is fully solved.

2. Curare possesses, in addition to its classical action on neuro-muscular transmission, an action on autonomic synaptic transmission which transmission it is capable of blocking.

3. The effects of a sufficient concentration of a curare on the central nervous system are a transient increase in activity, followed by decreased activity.

4. The above pharmacological actions of curare can be tentatively attributed to its effect upon cholinergic structures, whose ability to respond to acetylcholine is selectively and progressively modified.

5. The clinical use of curare, in shock-therapy and anesthesia, is thoroughly established. The full evaluation of the use of curare in spasticities awaits further experimentation.

6. The clinical use of curare for the treatment of conditions other than those involving the striated musculature is provocative of interest but must be viewed with cautious reserve.

REFERENCES

(1) McINTYRE, A. R. Curare, its history, nature, and clinical use. 1947, chapters 1–5.

(2) BOEHM, R. Chemische Studien über das Curare. Beitr. g. Physiol., Leipzig, 1886, 173–192.

(3) KING, H. Curare alkaloids. I. Tubocurarine. J. Chem. Soc. 1381, 1935.

(4) WINTERSTEINER, O. AND J. D. DUTCHER. Curare alkaloids from *Chondodendron tomentosum*. Science **97**: 467, 1943.

(5) BERNARD, C. Leçons sur les effets des substances toxiques et médicamenteuses. Paris, 1857, 316.

(6) KÜHNE, W. Ueber die Wirkung des amerikanischen Pfeilgiftes. Arch. f. Anat., Physiol. u. wissensch. Med., 477, 1860.

KÜHNE, W. Zur Histologie der motorischen Nervenendigung, Untersuch. a. d. physiol. Inst. Heidelberg **2**: 187, 1882.

KÜHNE, W. Über die Wirkung des Pfeilgiftes auf den Nervenstämme. Heidelberg, 1886.

(7) LANGLEY, J. N. On the contraction of muscle chiefly in relation to the presence of receptive substances. J. Physiol. **36**: 347, 1907. Ibid. **37**: 165, 1908. Ibid. **38**: 285, 1908. Ibid. **39**: 235, 1909.

(8) JUDÉE. Mode d'action du curare sur les cellules nerveuses dites motrices ou grosses cellules. Compt. rend. Soc. d. biol., 8.s., **1**: 292, 1884.

(9) CAREY, E. J. Effect of CO_2 on ameboid changes in motor nerve plates in intercostal muscle. Proc. Soc. Exper. Biol. and Med. **47**: 67, 1941.

CAREY, E. J. Pathologic effects of CO_2 and electricity on the explosive ameboid motion in motor nerve plates in intercostal muscle. Am. J. Path. **18**: 237, 1942.

CAREY, E. J. Experimental histopathology of motor end plates produced by quinine, curare, prostigmine, acetylcholine, strychnine, lead tetraethyl, and heat. Ibid. **20**: 341, 1944.

(10) KING, R. AND W. A. WILLARD. The effects of fatigue and curare on the morphology of the motor end plates. Anat. Rec. **91**: 286, 1945 (abstract).

(11) KÜHNE, W. Utersuchungen über Bewegungen und Veränderungen der contraktilen Substanzen. Arch. f. Anat., Physiol. u. wissensch. Med. 564–642, 1859.
(12) KUFFLER, S. W. Electric potential changes at an isolated nerve-muscle junction. J. Neurophysiol. **5**: 18, 1942.
(13) DAVIS, H. AND A. FORBES. Chronaxie. Physiol. Rev. **16**: 407, 1936.
(14) LAPICQUE, L. Neuromuscular isochronism and chronological theory of curarization. J. Physiol. **81**: 113, 1934.
(15) DALE, H. H., W. FELDBERG AND M. VOGT. Release of acetylcholine at voluntary motor nerve endings. J. Physiol. **86**: 353, 1936.
(16) MCINTYRE, A. R. Curare, its history, nature, and clinical use. Univ. of Chicago Press, 1947, chapter 8.
(17) DEHENNOT, O. Les poisons curarisants et la fatigue musculaire. Compt. rend. Soc. de biol. **117**: 318, 1934.
(18) SIMONART, A. AND F. E. SIMONART. L'acétylcholine et le muscle strié normal de mammifère, Arch. internat. de pharmacodyn, et de thérap. **49**: 302, 1935.
(19) SIMONART, A. Etude de l'action de l'acétylcholine sur le muscle strié de mammifère. Arch. internat. de pharmacodyn, et de thérap. **51**: 381, 1935.
(20) MCINTYRE, A. R., R. E. KING AND A. L. DUNN. Electrical activity of denervated mammalian skeletal muscle as influenced by d-tubocurarine. J. Neurophysiol. **8**: 297, 1945.
(21) BRISCOE, G. Antagonism between curarine and prostigmin and its relation to myasthenia gravis. Lancet **1**: 470, 1936.
BRISCOE, G. Depressant peripheral effect of prostigmin and its temporary antagonism by curarine. J. Physiol. **86**: 1P, 1936.
BRISCOE, G. The antagonism between curarine and acetylcholine. Ibid. **87**: 425, 1936.
BRISCOE, G. Anti-curare action of substance 36, closely related to prostigmin. Lancet **1**: 621, 1937.
BRISCOE, G. Eseriniform and curariform action of certain onium salts. J. Physiol. **93**: 6P, 1938.
BRISCOE, G. Changes in muscle contraction curves produced by drugs of the eserine and curarine groups. Ibid. 194–205.
BRISCOE, G. The antagonism between curarine and prostigmin. Lancet **1**: 469, 1936.
(22) ECCLES, J. C., B. KATZ AND S. W. KUFFLER. Effect of eserine on neuromuscular transmission. J. Neurophysiol. **5**: 211, 1942.
(23) WILSON, A. T. AND S. WRIGHT. Anti-curare action of potassium and other substances. Quart. J. Exper. Physiol. **26**: 127, 1937.
(24) MCINTYRE, A. R. AND R. E. KING. d-Tubocurarine chloride and choline esterase. Science **97**: 69, 1943.
(25) ECCLES, J. C., B. KATZ AND S. W. KUFFLER. Electric potential changes accompanying neuro-muscular transmission. Biol. Symposia **3**: 349, 1941.
(26) FENG, T. P. Studies on the neuro-muscular junction: universal antagonism between calcium and curarizing agencies. Chinese J. Physiol. **10**: 513, 1936.
(27) CHAO, J. Action of calcium and curare on muscular contraction and neuromuscular transmission. Chinese J. Physiol. **10**: 545, 1936.
(28) KUFFLER, S. W. Specific excitability of the endplate region in normal and denervated muscle. J. Neurophysiol. **6**: 99, 1943.
KUFFLER, S. W. The effect of calcium on the neuromuscular junction. J. Neurophysiol. **7**: 17, 1944.
(29) KELBLING, W. Protective action of parathyroid hormone against poisoning by curarine. Arch. f. exper. Path. u. Pharmakol. **195**: 567, 1940.
(30) WIRTH, F. Protective action of dihydrotachysterol against curarine poisoning. Arch. f. Exper. Path. u. Pharmakol. **195**: 564, 1940.
(31) WEST, R. A. A pharmacological study of derivatives of two specimens of *tubo*-curare,

and an examination of four members of genus *Strychnos*. Arch. internat. de pharmacodyn. et de thérap. **56:** 81, 1937.

(32) MIES, H. Curare und vegetative Innervation des Skelettmuskels. Ztschr. f. Biol. **98:** 70, 1937.

(33) FENG, T. P. Studies on neuro-muscular junction: the effect of guanidine. Chinese J. Physiol. **13:** 119, 1938.

(34) McINTYRE, A. R. Curare, its history, nature, and clinical use. 1947, chapter 15.

(35) SOLANDT, D. Y. AND J. W. MAGLADERY. The relation of atrophy to fibrillation in denervated muscle. Brain **63:** 255, 1940.

(36) FEINSTEIN, B., R. E. PATTLE AND G. WEDDELL. Metabolic factors affecting fibrillation in denervated muscle. J. Neurol. Neurosurg. and Psychiat. **8:** 1, 1945.

(37) BROWN, G. L. AND W. FELDBERG. Differential paralysis of the superior cervical ganglion. J. Physiol. **86:** 10P, 1936.

(38) McINTYRE, A. R. Curare, its history, nature, and clinical use. 1947, chapter 9.

(39) McINTYRE, A. R. Curare, its history, nature, and clinical use. 1947, chapters 9, 10, 11, 12, 13 and 14.

(40) BREMER, F., J. TITECA AND L. VAN. MEIREN. Sur la sensibilité au curare de la rigidité tétanique locale. Compt. rend. Soc. de biol. **97:** 895, 1927.

BREMER, F. AND J. TITECA. Nouvelles recherches sur le mécanisme de l'atone curarique. Compt. rend. Soc. de biol. **107:** 253, 1931.

BREMER, F. AND J. TITECA. Mécanisme de l'action électif du curare sur le tonus postural et les hypertonies. Ibid. **97:** 1407, 1927.

BREMER, F. AND J. TITECA. Action de l'adrénaline sur l'atonie musculaire du stade initial de la curarisation. Ibid. **99:** 624, 1928.

BREMER, F. AND J. TITECA. Atonie curarique et inhibition de Wedensky. Arch. internat. de physiol. **42:** 223, 1935.

(41) HARTRIDGE, H. AND R. WEST. Parathyroid tetany in dogs and its abolition by curare. Brain **54:** 312, 1931.

(42) BURMAN, M. S. Therapeutic use of curare and erythroidine hydrochloride for spastic and dystonic states. Arch. Neurol. and Psychiat. **41:** 307, 1939.

BURMAN, M. S. Curare therapy for the release of muscle spasm and rigidity in spastic paraylsis and dystonia musculorum deformans. J. Bone and Joint Surg. **20:** 754, 1938.

BURMAN, M. S. Clinical experiences with some curare preparations and curare substitutes. J. Pharmacol. and Exper. Therap. **69:** 143, 1940.

BURMAN, M. S. Curare therapy. J. A. M. A. **129:** 1286, 1945.

(43) HARVEY, A. M. AND R. L. MASLAND. Actions of curarizing preparations in the human. J. Pharmacol. and Exper. Therap. **73:** 304, 1941.

(44) SCHLESINGER, E. B. Use of curare in oil in treatment of spasticity following injury of the spinal cord. Arch. Neurol. and Psychiat. **55:** 530, 1946.

(45) DENHOFF, E. AND C. BRADLEY. Curare treatment of spastic children. New England J. Med. **226:** 411, 1942.

(46) WEST, R. The pharmacology and therapeutics of curare and its constituents. Proc. Roy. Soc. Med. **28:** 565, 1935.

(47) JOSEPH, D. R. AND S. J. MELTZER. On the difference between the effects of intravenous and intra-aortic injections of curarin in frogs. J. Pharmacol. and Exper. Therap. **3:** 465, 1911–12.

(48) STERN, L. AND E. ROTHLIN. Effects of the local application of curare upon different parts of the cerebellum. Arch. Sc. Phys. Nat., Suppl. 11–19, **4:** 45, 1918.

STERN, L. AND E. ROTHLIN. Du effets de l'application directe du curare sur les différentes parties du cervelet. Compt. rend. Soc. de phys. et d'hist. nat. **35:** 11, 1918.

STERN, L. AND E. ROTHLIN. Effets de l'application directe du curare sur les différentes parties du cervelet. Schweiz. Arch. f. Neurol. u. Psychiat. **3:** 234, 1918.

(49) HARLOW, H. F. AND R. STAGNER. The effect of complete striate muscle paralysis on the learning process. J. Exper. Psychol. **16:** 283, 1933.

HARLOW, H. F. The effects of incomplete curare paralysis upon formation and elicitation of conditioned responses in cats. J. Genet. Psychol. **56:** 273, 1940.

(50) GIRDEN, E. AND E. CULLER. Conditioned response in curarized striate muscle in dogs. J. Comp. Psychol. **23:** 261, 1937.

GIRDEN, E. Cerebral mechanisms in conditioning under curare. Am. J. Psychol. **53:** 397, 1940.

GIRDEN, E. Generalized conditioned responses under curare and erythroidine. J. Exper. Psychol. **31:** 105, 1942.

GIRDEN, E. Dissociation of pupillary conditioned reflexes under erythroidine and curare. Ibid. 322–32.

(51) FEITELBERG, S. AND E. P. PICK. Action of curare on the brain of the frog. Proc. Soc. Exper. Biol. and Med. **49:** 654, 1942.

PICK, E. P. AND K. UNNA. The effect of curare and curare-like substances on the central nervous system. J. Pharmacol. and Exper. Therap. **83:** 59, 1945.

(52) MCINTYRE, A. R., A. L. DUNN AND P. E. TULLAR. The effect of d-tubocurarine on the electrical activity of dogs' brains. Federation Proc. **5:** No. 1, 67, 1946.

(53) BENNETT, A. E., A. R. MCINTYRE AND A. L. BENNETT. Pharmacologic and clinical investigations with crude curare, J. A. M. A. **114:** 1791, 1940.

(54) GRIFFITH, H. R. AND G. E. JOHNSON. The use of curare in general anesthesia. Anesthesiology **3:** 418, 1942.

(55) GRIFFITH, H. R. The use of curare in anaesthesia and for other clinical purposes. Canad. M. A. J. **50:** 144, 1944.

GRIFFITH, H. R. Curare in anesthesia. J. A. M. A. **127:** 642, 1945. Also Lancet **2:** 74, 1945.

GRIFFITH, H. R. Curare in anesthesia (reply). J. A. M. A. **127:** 644, 1945.

GRIFFITH, H. R. Curare—a new tool for the anaesthetist. Canad. M. A. J. **52:** 391, 1945.

(56) CULLEN, S. C. The use of curare for improvement of abdominal muscle relaxation during inhalation anesthesia. Surgery **14:** 261, 1943.

CULLEN, S. C. Clinical and laboratory observations on the use of curare during inhalation anesthesia. Anesthesiology **5:** 166, 1944.

CULLEN, S. C. The use of curare in anesthesia. South. M. J. **38:** 144, 1945.

(57) GROSS, E. G. AND S. C. CULLEN. The effects of anesthetic agents on muscular contraction. J. Pharmacol. and Exper. Therap. **78:** 358, 1943.

(58) WHITACRE, R. J. AND A. J. FISHER. Use of curare in anesthesia. Ohio State M. J. **40:** 1155, 1944. Also: Anesthesiology **6:** 124, 1945.

(59) SMITH, S. M. Symposium on curare. A. M. A. session, San Francisco, J. A. M. A. **131:** 1148, 1946.

(60) MCINTYRE, A. R. Curare, its history, nature, and clinical use. 1947, chapter 14.

(61) PERLSTEIN, M. A. AND A. WEINGLASS. Fatal effects of prolonged complete curarization. Am. J. Dis. Child. **67:** 360, 1944.

(62) RANSOHOFF, N. S. Curare in the acute stage of poliomyelitis: preliminary report. J. A. M. A. **129:** 129, 1945.

(63) BENNETT, A. E., A. R. MCINTYRE AND P. T. CASH. Comparison of curarization with myasthenia gravis. Exhibit, A. M. A., J. A. M. A. **131:** 321, 1946.

(64) MCINTYRE, A. R., P. TULLAR AND M. FRANK. Failure of neostigmine as an antidote for curare. Proc. of the Cent. Soc. for Clinical Research **19:** 73, 1946.

(65) MCINTYRE, A. R. Curare, its history, nature, and clinical use. 1947, chapter 14.

THE URINARY COPROPORPHYRINS IN HEALTH AND DISEASE[1]

CECIL JAMES WATSON AND EVREL A. LARSON

Department of Medicine, University of Minnesota Hospital, Minneapolis

The literature relating to the urinary porphyrins has become so extensive that it would scarcely be feasible to review it comprehensively in the space which has been allotted to the present paper. The authors have striven rather to bring together the essential pieces of information which comprise the existing knowledge of the subject. A number of reviews have appeared to which the reader is referred for additional orientation to the literature, both with respect to the urinary porphyrins (9d; 16i; 99c), and to porphyrin metabolism in general (9c; 11; 16i; 24e, k; 32a, b, d; 58b; 73b; 78b; 97c; 100g, h). Because of space restriction, the present review will concern itself mainly with the urinary coproporphyrins. Other porphyrins and other aspects of the coproporphyrin problem will be considered only as they relate to the coproporphyrins of the urine.

The term uro- and coproporphyrin have proven somewhat unfortunate in the light of knowledge of these substances gained since Hans Fischer first described and named them. Prior to Fischer's studies, the naturally occurring porphyrins had been generally assumed to be the hematoporphyrin as first prepared by Hoppe-Seyler (41) and later, in crystalline form, by Nencki and Sieber (64). Reasons for this confusion will appear presently. Fischer's first observations were made in a case of congenital porphyria, the famous case Petry, in which the urine, as it characteristically does in this disease, contained large amounts of uro- and relatively small amounts of coproporphyrin (24a). The feces from this case was found to contain only coproporphyrin (24b), and in fact, the presence of uroporphyrin in the feces of porphyria cases was overlooked until relatively recently (80a). Fischer's isolation of crystalline uro- and coproporphyrin from urine and feces, respectively, initiated a brilliant series of studies of the porphyrins which were terminated by his untimely death in 1945. These studies of Fischer and his school have provided much of the existing knowledge concerning the structure and chemical interrelationships of the porphyrins. Relatively early in the course of this long research, Fischer was able to show that the configuration of the uro- and coproporphyrin isolated from the case Petry, differed from that of the protoporphyrin in the hemoglobin molecule (24e). This difference may be noted in figure 1, in which it is seen that there is opposite placement of the methyl and propionic acid groups on pyrrol nucleus IV (left lower). Fischer synthesized the four "aetioporphyrins", which have only methyl and ethyl groups on the porphyrin ring (24e, k). These do not occur in nature, but they serve as the basis for his classification, in that all other porphyrins belong to one of four main types, depending upon correspondence of the side groups with the methyl or ethyl groups of the aetioporphyrins. Thus the uro- and copro-

[1] Aided by grants from the John and Mary R. Markle Foundation, New York City, and the Medical Research Fund of the Graduate School, University of Minnesota.

porphyrin isolated from the porphyria case were found to have the configuration of aetioporphyrin I, while the protoporphyrin of the hemoglobin molecule was later shown to have the type III structure[2] (24p). Fischer thus revealed the "dualism" of the porphyrins in nature. Although theories of porphyrin synthesis in the animal organism have been proposed which postulate a minute formation of type II or IV porphyrins (16g, i; 94), they have never been isolated from natural material nor has any evidence been described to indicate their occurrence.

As will be discussed subsequently, the formation and excretion of coproporphyrins I and III, varying in relative and absolute amounts under different circumstances, has been well established. Their fundamental significance, however, is but poorly understood. The formation and excretion of uroporphyrin I has come to be recognized as a characteristic feature of the metabolic error

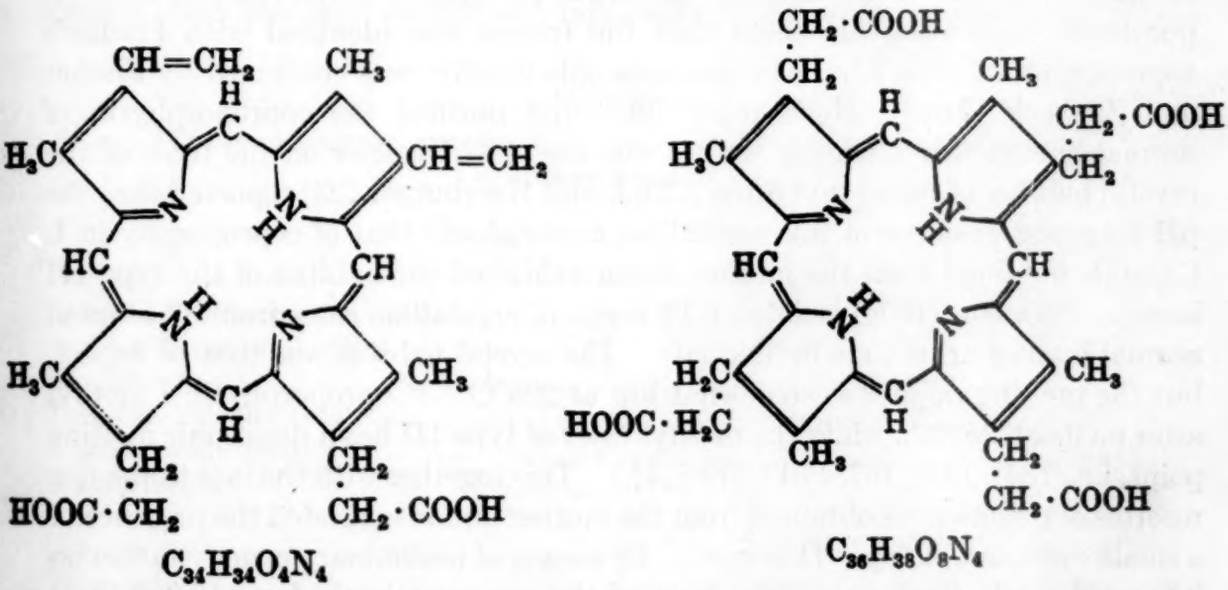

Fig. 1. Structural formulae of coproporphyrin I and of protoporphyrin 9 (isomer type III), according to H. Fischer (24d).

which constitutes porphyria, in contradistinction to the idiopathic or secondary coproporphyrinurias. The position of uroporphyrin III in the porphyrin scheme, is much less secure. Recent studies (32c; 100q) have shown that what has formerly been designated as uroporphyrin III is actually a mixture of uroporphyrin I with a type III porphyrin, the latter, however, not having the elementary composition of a uroporphyrin isomer. The uroporphyrin problem would require separate consideration from many standpoints, and except where it relates in an intimate way to the urinary coproporphyrin, it will not be included in the ensuing review.

[2] Actually this is designated as protoporphyrin 9, since it corresponds in configuration with mesoporphyrin 9. Fischer further classified porphyrins having more than two types of side groups, on the basis of correspondence in configuration with one of the 15 mesoporphyrins. The latter have four methyl, two ethyl, and two propionic acid side groups.

The physical constants of the coproporphyrins and their metal complexes and esters, including melting points, crystal habitus, absorption and fluorescence spectra, and solubilities have been given in detail elsewhere (16i; 24k; 49; 50; 100h).

The coproporphyrin of normal urine. MacMunn (56), Saillet (76) and Garrod (27a, b, c) were the first to recognize the presence of a porphyrin in normal urine. These earlier investigators labored under the difficulty of rather crude spectroscopes with which it was scarcely possible to distinguish between copro- and hematoporphyrin, the absorption spectra of which differ by not more than 10 Å (24k). Thus it is not surprising that the porphyrin of normal urine, as well as that found in increased amounts in a variety of pathological states, was believed for many years to be the hematoporphyrin which Nencki and his coworkers first established as a well defined chemical entity (64). Schumm (79a, b) recognized the differing absorption spectra of the urinary porphyrin and hematoporphyrin, indicating his belief that the former was identical with Fischer's coproporphyrin (24a, b). This spectroscopic identity was confirmed by Fischer and Zerweck (24m). Hoerburger (39a) first purified the coproporphyrin of normal human urine, stating that it was the type I isomer on the basis of the crystal habitus of its methyl ester. Fink and Hoerburger (23) reported that the pH fluorescence curve of this crystalline material was that of coproporphyrin I. Crystals obtained from the mother liquor exhibited the habitus of the type III isomer. Watson (100e) isolated 0.13 mgm. of crystalline ester from 33 liters of normal human urine (one individual). The crystal habitus was that of type I, but the melting point was somewhat low at 228°C. (Coproporphyrin I methyl ester melts at 250°C., while the methyl ester of type III has a dimorphic melting point, i.e., 135°, 144°, 167–170°C. [24i, k].) This together with the fact that a few rosettes of prisms were obtained from the mother liquor suggested the presence of a smaller amount of type III isomer. By means of preliminary concentration on infusorial earth, Grotepass (33b) isolated the coproporphyrin from 10,000 liters of presumably normal human urine, pooled from a large number of individuals. Upon fractional crystallization of the yield, which was approximately 200 mgm., 96 mgm. of type I and 87 mgm. of type III were obtained. As pointed out by Dobriner and Rhoads (16i), the chief objection to Grotepass' conclusion that this is the isomer distribution of normal human urine, is the possibility of inclusion in the pool of urine from an individual having a latent or symptomless type III porphyrinuria. It will be noted subsequently that such a condition may easily be overlooked. Furthermore, it was possible that chemical or metal exposure, or alcoholism (vide infra) was present in at least some of the individuals contributing to Grotepass' pool. It was desirable, therefore, to know the isomer distribution in a series of urines for each of which it had first been determined that the concentration of total coproporphyrin was within the normal range (vide infra). Data of this type have recently been gained (100s) in a study of 30 normal individuals, employing the differential precipitation "fluorescence quenching" technique (80b, e). The range of isomer distribution in this study was from 65 to 92 per cent type I, and from 8 to 35 per cent type III. These 30 individuals

represented both sexes about equally, but were all below 30 years of age, the majority being students between 20–25. An additional group of 11 individuals of both sexes between the ages of 69–82 were also studied (100s). This revealed a slightly higher relative percentage of type III isomer. It appears, however, that this apparent increase is due to a mild decrease of type I. The range of values for the type I and type III isomers in the young and old individuals was as follows:

	YOUNG	OLD
I	16.—89 γ per day	14.7—57 γ per day
III	1.4—31.7 γ per day	4.5—34.3 γ per day

The concentration and per diem output of the total coproporphyrin in normal human urine has been determined repeatedly in the past, the reported values

TABLE 1

The average normal excretion of coproporphyrin in the urine as determined by various investigators

AUTHOR	URINARY COPROPORPHYRIN IN γ PER 24 HRS.
Franke and Fikentscher (25b)	10–30
van den Bergh et al. (96c)	10–100
Brugsch (9a, d)	4–50
Tropp and Siegler (93a)	18–110
Vigliani et al. (98d)	6–46
Vannotti (97a)	10–80
Carrié (11)	0–60
Lageder (52)	0–100
Thiel (92a)	0–100
Dobriner et al. (16n)	41–120
Watson et al. (100s)	14–99
Mason and Nesbitt (58a)	0–100
Franke and Litzner (25c)	3–7 (per 100 cc.)

ranging from 1 to 8 γ per 100 cc. (25c; 100s; w), and from 0 to 120 γ per day. The results of a number of investigators, as obtained with a variety of methods (vide infra), are shown in table 1.

The methods of determination have been, in the main, fluorimetric, depending upon the characteristic red fluorescence of the porphyrins in Wood's light. In accordance with Stoke's rule, the excited fluorescent light is of longer wave length than the exciting light which is absorbed (5a). For the coproporphyrins in 1 per cent HCl the exciting light is 4010Å (max.) (80f), while in 5, 10, 25 per cent HCl, the exciting wave lengths are 4018, 4030, and 4058 Å (max.) respectively (79d). Fluorescence is excited to a lesser extent by light of 5480 Å (max.). The fluorescence spectral band is found between 6200–6300 Å, maximum at 6250 Å

(51b.). The fluorescence intensity has been measured in various ways including the Pulfrich stufenphotometer (22; 25b; 97c; 98d), or fluorophotometers such as the Pfaltz-Bauer (58a) Klett (100x), or specially constructed types (63; 96a). Spectrometric (16f; 78f; 79c), and colorimetric methods (16n; 92a, b) have also been employed. Fluorescence methods are considerably more sensitive and require smaller starting volumes of urine.

In the study of isomer distribution in 41 normal individuals, as referred to in the above, the 24 hour quantity of total corproporphyrin was likewise determined in each instance. The range was from 14–99 γ. The statistical significance of these findings is discussed in more detail elsewhere (100s). On the basis of this study, it is believed that the upper limit of normal is 100 γ per 24 hours. The principal sources of error contributing to higher values obtained with colorimetric methods, as reported by others (20; 92b) are thought to be the measurement of impurities which are included due to insufficient preliminary purification. Thus it has been noted repeatedly that unless the porphyrin is taken back and forth twice between ether and HCl, unduly high values result, especially when the method depends upon absorption. Inadequate purification leads to unduly high values with colorimetric techniques and low values with those based on fluorimetry, in the former because of increased absorption of light, and in the latter because of masking of fluorescence, by impurities. Thus the method of Tropp and Siegler (93a) employs a continuous extraction apparatus which entails prolonged heating in boiling ether, together with any other impurities extracted from the urine. Experience[3] with continuous ether extraction has revealed consistent loss, as contrasted directly with repeated extraction in a separatory funnel. Nevertheless, Tropp and Siegler's range of normal values is slightly higher than ours, as noted in table 1. The reason for this is not apparent.

Poulikakos and Tropp (69) found little or no relation of the per diem amount of porphyrin to the volume of urine, in normal individuals. With increased excretion of porphyrin, however, relative increases were observed with larger volumes of urine.

Liver disease and jaundice. Garrod (27a, b, c) emphasized the occurrence of increased amounts of porphyrin, believed by him to be hematoporphyrin, in the urine in cases of liver disease. This has been confirmed by many subsequent investigators (9d; 16a; 24o; 34b; 65; 96c; 100b, t, u). Fischer and Zerweck (24o) showed that it was coproporphyrin which was increased, but did not determine the preponderant isomer type. van den Bergh (96c) found that coproporphyrin appeared in the serum and was increased in amount in the urine, both in cases of liver disease and of mechanical or obstructive jaundice. He postulated that this was due simply to a faulty excretion by the liver of the coproporphyrin normally present in the bile (96c). As will be discussed in more detail later, van den Bergh (96c) believed that this coproporphyrin is derived from the protoporphyrin of the erythrocytes, the conversion from one to the other being regarded as a normal function of the liver. Although later studies (77; 98a;

[3] Unpublished.

100n) have not supported this belief, the finding has been generally confirmed that coproporphyrin is regularly increased in the urine of cases of regurgitation jaundice whether due to diffuse liver damage or to biliary obstruction (9d; 16a, i; 65; 100b, t, u). Thus a recent study (100o, t, u) has revealed a significant elevation, usually from two to four times the upper limit of normal, in a series of cases of mechanical jaundice, and of jaundice due to hepatitis and cirrhosis. van den Bergh (96c) observed somewhat higher values in mechanical than in parenchymal jaundice, but did not attempt to employ the urinary coproporphyrin value in a differential diagnostic way. The data recently reported (100o) are

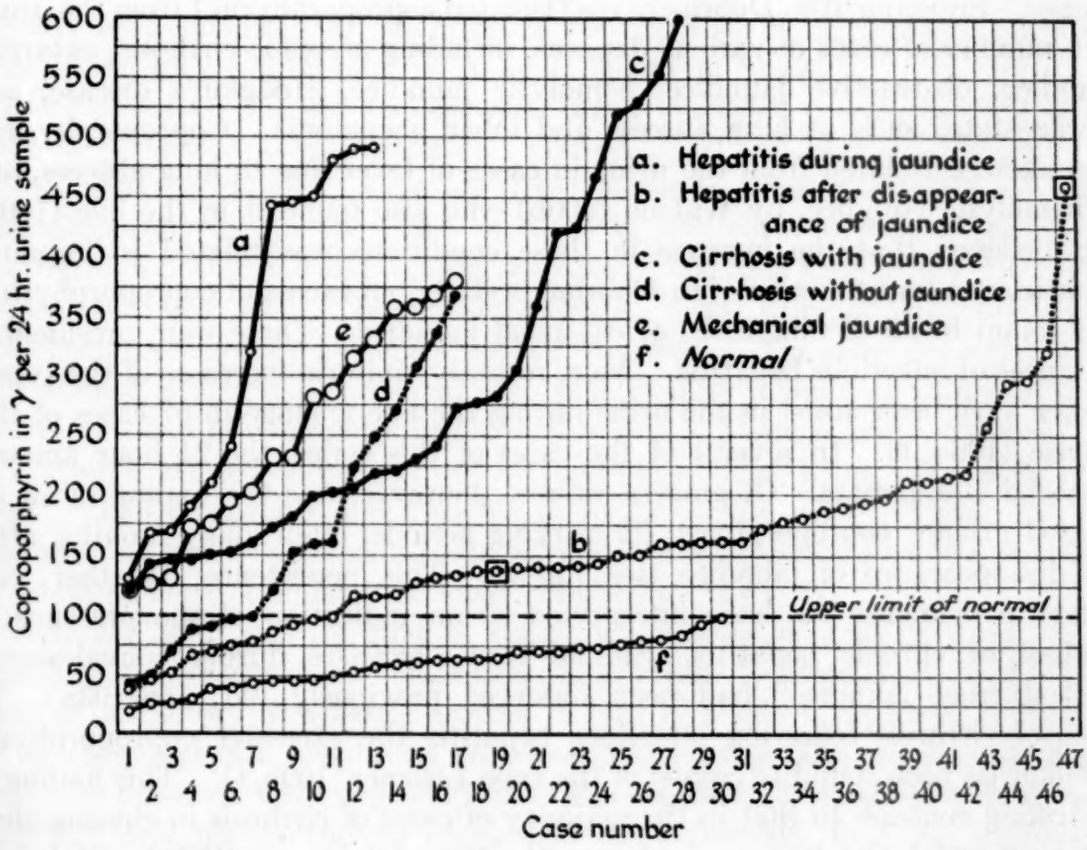
Coproporphyrin in γ per 24 hr. urine sample
600
550
500
450
400
350
300
250
200
150
100
50
0
a. Hepatitis during jaundice
b. Hepatitis after disappearance of jaundice
c. Cirrhosis with jaundice
d. Cirrhosis without jaundice
e. Mechanical jaundice
f. Normal
Upper limit of normal
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46
Case number

Fig. 2. Total coproporphyrin in cases of hepatitis and cirrhosis. Case 19 in group b was studied about 18 months after the acute attack of infectious jaundice; case 47 in this group about 3 months afterward.

shown in figure 2. It is evident from these and additional data which are discussed in separate communications (100t, u) that the total coproporphyrin determination has relatively little value in the differential diagnosis of jaundice.

Increase of the urinary coproporphyrin has been referred to frequently as a sensitive index of liver functional impairment (25a, b; 65; 93a). After alcohol ingestion, Franke (25a, b) observed prompt sharp increases which averaged 100 per cent, following one liter of beer or 90 cc. of cognac. Significant and rather uniform increases were also recorded by Franke in cases of "catarrhal" jaundice, subacute atrophy of the liver, cirrhosis of the liver, mechanical jaundice, and passive congestion of the liver. Franke noted generally higher values in

parenchymal than in mechanical jaundice. He did not regard the high values observed in 45 cases of lead poisoning as related to liver injury, as he found no evidence of the latter from the standpoint of other investigations. This matter will be considered in more detail later.

The first determination of isomer type in an instance of liver disease was carried out on urine from a case of cirrhosis of the liver which followed the use of cinchophen (100e). From the standpoint of the effect of chemical toxicity on porphyrin metabolism (vide infra), it may be noted, however, that a considerable interval of time had elapsed in this case, between the ingestion of the cinchophen and the time of isolation of the porphyrin. The latter proved to be the type I isomer. Subsequently, Dobriner (16a) isolated coproporphyrin I from the urines of a number of cases of various diseases, including atrophic cirrhosis, catarrhal jaundice, obstructive jaundice, hemolytic jaundice, Hodgkin's disease, and febrile states such as lung abscess and lobar pneumonia. Coproporphyrin I was likewise isolated from the urine in cases of fever due to lung abscess, and in hemolytic jaundice, by Watson (100b) who also found it in the bile (100f), and believed that the increase in these conditions was related to impaired hepatic excretory function. In Dobriner's study, an excess of coproporphyrin I was found in all of nine cases of catarrhal jaundice. These were presumably instances of infectious hepatitis. More recently, uniform increases of the type I isomer have been noted in the urine during the icteric stage in 57 cases of this disease (100o, t). In several of the cases of this series, the 24 hour amount exceeded 400 gamma. Of greater interest, however, was the finding of an increased urinary coproporphyrin for varying periods, often many months, after the disappearance of jaundice (see fig. 2). This persistence, together with collateral evidence has been interpreted as an indication of the presence of residual or chronic hepatitis. Similar slow decreases during convalescence of "catarrhal jaundice" had been observed previously (9d; 16a; 93a). In cases of cirrhosis following infectious hepatitis the excessive coproporphyrin has likewise been found to consist of the type I isomer (100o, t). This finding is in striking contrast to that in the majority of cases of cirrhosis in chronic alcoholics, in which the increase is due to the type III isomer (100u). Dobriner had previously reported an excess of type I in 6 cases of "atrophic cirrhosis," and of type III in one such case, but without stating which, if any of these instances, were chronic alcoholics.

The effect of alcohol on porphyrin metabolism has not yet received sufficient study. This problem will be dealt with now because of its close relation to that of hepatic cirrhosis, rather than in the later section dealing with chemical toxicity. Franke's finding of a sharp increase of coproporphyrinuria in acute experiments, has already been cited. This was confirmed by Brugsch and Keys (9f) who also noted increases in chronic alcoholism and isolated the type I isomer in two cases and the type III in one. Watson (100i) isolated coproporphyrin III from the urine in alcoholic pellagra (vide infra). In a study which is still in progress and which will be reported in detail elsewhere (90), it has been found that the great majority of chronic alcoholics excrete excesses of type III isomer while drinking

but usually exhibit a normal porphyrin excretion within a short time, often indeed a few days after complete abstinence from alcohol. Thus it is of considerable interest that an increased coproporphyrin III excretion persists in many instances of cirrhosis, in chronic alcoholics, long after the ingestion of alcohol has stopped. It appears, in other words, that some peculiar factor is operative in these cases which may relate not only to the persistence of the type III coproporphyrinuria, but also to the development of cirrhosis. Illustrative data are presented in separate communications (90; 100u).

Dobriner (16a) found coproporphyrin III in a case of hemochromatosis. In unexplained contrast to this finding, an excess of coproporphyrin I has recently been observed in the urine of two cases of hemochromatosis (100u). This discrepancy will be referred to again in the following.

Blood diseases. Fischer and Zerweck (24m) identified coproporphyrin in the urine of *pernicious anemia* patients. Garrod (27b) had previously referred to this as hematoporphyrin. Günther (34b), however, failed to observe increased "hematoporphyrin" in the urine in cases of pernicious anemia. The coproporphyrin in both urine and feces in this disease has been shown to be the type I isomer (16h; 98c; 100f). Inconstant increases in the urine of moderate degree have been discussed by Dobriner and Rhoads (16g). The same is true in *hemolytic anemia*, which, like pernicious anemia in relapse, is characterized by considerable increases of coproporphyrin I in the feces. There is some evidence that the latter is related to the rate of erythropoiesis (16d, h, m; 80f; 100f) (vide infra). The inconstant and variable increases in the urine in both of these diseases may well be analogous to the variable urobilinogenuria which is believed to relate to hepatocellular functional impairment (100a, f).

Vannotti (97c) was the first to report an increase in urinary porphyrin in a case of *aplastic anemia.* Dobriner, Rhoads and Hummel (16j), studied six cases of refractory or aplastic anemia in four of which an increase was found to be composed of a mixture of coproporphyrin I and III. In two additional cases, the presence of the type III isomer was suggested, but could not be proven. Dobriner and his co-workers considered the possibility that the increase of coproporphyrin III in these cases indicated a toxic or extrinsic chemical factor in their etiology. The relation of chemicals and heavy metals to type III coproporphyrinuria will be discussed in more detail in a later section. Recent studies of urinary coproporphyrin excretion in 12 cases of refractory or aplastic anemia, carried out by means of the differential precipitation technique (80b, e) have revealed a range of total coproporphyrin of from 119–363 γ per 24 hours, of which 56–92 per cent was the type III isomer. These data will be presented in detail in a separate communication (100v).

Inconstant mild increases of urinary coproporphyrin in leukemia have been reported in the literature and are discussed by Dobriner (16b, i). Dobriner (16b) found increases in cases of Hodgkin's disease ranging from 200 to 600 micrograms in febrile cases, shortly before death. This was ascertained to be the type I isomer in 6 instances. It was therefore of particular interest that in the course of a more recent study (100v), relatively large amounts of copro-

porphyrin III were encountered in three cases of Hodgkin's disease, while in two others, the excess was the type I isomer. This difference has not been explained; it will be referred to again in the discussion of origin and significance of the urinary coproporphyrins.

Hemorrhage and the effect of blood in the gastrointestinal tract. Duesberg (17a), studying hemorrhage in rabbits, did not observe any increase in urinary porphyrins. Dobriner and Rhoads (16h) found an increase in total coproporphyrin excretion in dogs, after bleeding from the jugular vein; the percentage of this increase, if any, excreted in the urine, was not stated. Dobriner's interpretation of this finding will be discussed later.

Increases of urinary porphyrin have repeatedly been ascribed to the presence of blood in the intestinal tract (6; 9a, b; 24h; 79c; 86a; 97c). The inconstant character of these increases induced Kaunitz (46) to administer hemoglobin, in 20 gram quantities, to normal individuals and to patients with various disorders, including liver disease. It was found that negligible, if any, increase occurred in the former, but that significant elevations were common in the latter group, especially in the cases with liver disease. Kaunitz interpreted this to indicate a formation of the type III isomer in the intestinal tract followed by partial absorption into the portal circulation with disposition by the normal liver, so that little or none was allowed to enter the general circulation and appear in the urine, while in the case of the damaged liver variable fractions gained access to the urine. The study of Jakob (42) offered indirect support for this belief. According to this study certain bacteria are capable of elaborating coproporphyrin III from hemoglobin, in vitro. Kämmerer (43a) had previously demonstrated the bacterial formation of protoporphyrin from hemoglobin, in vitro, but he believed that this transition required a symbiotic growth of anaerobes with E. coli, while others have shown that symbiosis is unnecessary, and that in the presence of hemoglobin, pure cultures of E. coli are capable of the elaboration of protoporphyrin (38a) and of coproporphyrin III (42).

The question of the relation of blood in the gastrointestinal tract, and of the bacterial flora of the intestine, to the urinary coproporphyrin, under normal and pathological conditions, has been studied in some detail in this laboratory (53a, b). Jakob's findings have been confirmed including the observation that certain strains of bacteria can synthesize coproporphyrin III in the absence of any demonstrable heme pigments or porphyrins, but in the presence of protein. It was not elaborated in synthetic media of the Gladstone type. In this connection it may be noted that Coulter and Stone (13) observed formation of coproporphyrin by diphtheria bacilli in bouillon cultures. The rate of formation appeared to be proportional to the toxin production. The isomer type was not determined.

Nevertheless, the recent study just referred to failed to support Kaunitz' view that hemoglobin in the intestinal tract causes increased coproporphyrinuria, even in the presence of liver functional impairment. No significant increases were observed in several instances of outspoken liver disease, following feeding of hemoglobin in amounts as large or larger than those which Kaunitz gave. It

is believed of even greater significance that there was repeated failure of absorption of coproporphyrin III given by mouth, after administration of 3–5 mgm., together with lack of increase of the coproporphyrinuria in such experiments. Thus it would appear that while the bacterial flora of the colon may produce small amounts of coproporphyrin III even in the absence of hemoglobin, the presence of this porphyrin in the bowel bears little or no relation to the amount in the urine. The results of this study, in fact, strongly indicate that the coproporphyrin III of human urine is a truly endogenous metabolite. Whether this applies as well to the type I isomer is not certain. This problem will be considered later (vide infra), but it may be noted at this point, that in one experiment in which 3 mg. of coproporphyrin I was administered orally, no increase was observed in the urine and approximately the amount given was recovered in the feces.

There is no evidence that chlorophyll in the diet contributes to the coproporphyrin of the urine. Brugsch (9d, g) has discussed this problem in detail.

Fever and infectious disease. The finding of coproporphyrin I in febrile states associated with pneumonia (16a) and lung abscess (16a; 100e) has already been referred to, and the possibility was considered that these increases were related to hepatic functional impairment.

More recently Cartwright and co-workers (11b) have observed increased coproporphyrinuria regularly in association with the anemia of pyogenic infection. The isomer type was not stated.

The uniform increase of coproporphyrin I in the urine of cases of infectious hepatitis during the icteric stage of the disease, and in many instances persisting after disappearance of jaundice, has also been mentioned. It was therefore an unexpected finding of much interest that the urine of cases of *acute poliomyelitis* regularly contained significant increases of type III isomer (100r). In general, larger amounts of total coproporphyrin, ranging up to 1000 gamma per day with 60–90 per cent of coproporphyrin III, were encountered in the more seriously ill patients, especially the bulbar type, but a strict correlation with severity or type has not been determined. Of 64 cases of the disease, 56 exhibited amounts of total coproporphyrin in excess of 100 gamma. In some instances in which the total coproporphyrin value was normal, the percentage of the type III isomer was abnormally high. Figure 3 shows the total coproporphyrin values and isomer distribution in 51 cases, as compared with the normal range. In a number of these instances crystalline coproporphyrin III methyl ester was isolated, thus confirming the results obtained by means of the differential precipitation technique (80b, e). The cause of this increase is unknown. Klüver (51a, b) established the presence of small amounts of coproporphyrin in the white matter of the central nervous system of warm blooded animals, and identified it tentatively as the type III isomer (51c). Based on studies of bacterial metabolism, Granick and Gilder (31) suggest that coproporphyrin may function as a regulator for the rate of oxygen consumption by living cells. It remains to be determined whether the coproporphyrin of the central nervous system has such a rôle. Studies by Chu and Watson (12)

have also tentatively identified the porphyrin as coproporphyrin III and have established the concentration at about 0.02–0.03 gamma per gram of human brain. It was found impossible to increase the concentration in rabbit brains by poisoning the animals with lead, in spite of the fact that a 10 or 20 fold increase of urinary coproporphyrin III was effected (12). Nor was it possible to achieve any increase in concentration in dogs' brains by injecting relatively large amounts of coproporphyrin III into the internal carotid artery one-half hour before the animals were killed and the brains examined (12). These observations render much more difficult the correlation of the above mentioned urinary findings in poliomyelitis with the central nervous system coproporphyrin; nevertheless, there may be a relationship and this possibility is receiving further study at the present time.

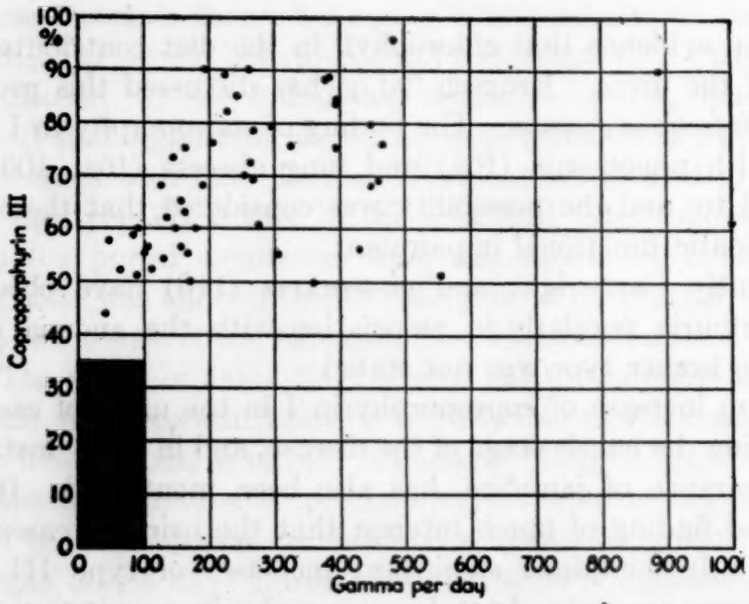

Fig. 3. Total coproporphyrin and per cent of type III isomer in 64 cases of poliomyelitis. The shaded area in the lower left corner represents the normal range. From the paper "Coproporphyrinuria (type III) in acute poliomyelitis," by C. J. Watson, Wm. Schulze, Violet Hawkinson, and A. B. Baker, Proc. Soc. Exper. Biol. and Med., **64**: 73, 1947. Reproduced by permission of the publishers.

The fact that two virus diseases such as infectious hepatitis and acute poliomyelitis are sharply differentiated on the basis of the urinary coproporphyrin isomer distribution, indicates that other virus diseases should be investigated in this regard. A study is in progress which will embrace both neurotropic virus affections such as encephalitis and Guillain-Barre' radiculitis, and those which are not neurotropic, such as atypical pneumonia, psittacosis and others. At the present time, the literature does not contain any data of this type. It is of interest, however, that in another disease of the brain, namely schizophrenia, Libowitzky and Scheid (55) report that the febrile episodes which are often observed, were characterized by an increased urinary coproporphyrin type I.

Kapp and Coburn (44a) described an increase of urinary coproporphyrin in acute rheumatic fever, thus giving emphasis and definition to the observations of

MacMunn on urohematin (56), and of Garrod (27a) and Stokvis (86b) on hematoporphyrin, many years earlier. Kapp and Coburn were able to isolate coproporphyrin III from the pooled samples of a number of cases. Kapp (44b) noted that the coproporphyrin was excreted in considerable part as the zinc complex. In several instances "sudden fatalities were preceded by intense porphyrinuria." In addition Kapp and Coburn state "the excretion of abnormally large amounts of porphyrin in acute rheumatism bore no relation to fever, haematuria, or the use of therapeutic measures per se." No mention was made in these papers of the use of salicylates or amidopyrine, although Dobriner and Rhoads (16i) state that Kapp and Coburn's patients were receiving these drugs at the time of the porphyrin isolation. Brownlee (8a) showed that amidopyrine, amongst other drugs of the coal tar group, produced coproporphyrinuria type III in rats. So far as can be determined, the effect of salicylates on porphyrin excretion has not been investigated. Watson and Hawkinson[4] have found moderate and variable increases of urinary coproporphyrin in acute rheumatic fever. These increases, however, were composed in all instances of the type I, rather than the type III isomer. The possibility was considered that the basis of the increase was a febrile or toxic effect on the liver. The administration of salicylates was without definite effect on the porphyrin excretion, no increases of type III isomer being observed.

Porphyria and idiopathic coproporphyrinuria. As noted at the outset, the entity of coproporphyrin was first established by H. Fischer who isolated it, together with uroporphyrin, from the urine of Petry, the celebrated case of porphyria (24a). This coproporphyrin was subsequently classified as the type I isomer (24e). Coproporphyrin III was likewise first obtained from the urine of a case of porphyria of the congenital, or light sensitizing variety (24e, f; 96a). Experience has revealed, however, that this latter finding is somewhat exceptional and that it is more common to find the type III isomer in the urine of the so-called acute type, i.e., that which is characterized by abdominal or nervous manifestations, while the type I isomer is most often encountered in the light sensitive variety, such as represented by the case Petry. The idea has even been advanced (62; 78a) that a strict chemical separation of the two types might be made on a chemical basis, but it is evident that there are too many exceptions, instances in which coproporphyrin I was isolated from the abdominal or nervous type (11a; 24j; 101), and coproporphyrin III from the light sensitive type, both in human cases (24f; 96d), and in cattle (73a, e), or, as in certain cases, both isomers in approximately equal amount (16b; 96a). Thus the case of van den Bergh and Grotepass (96a) was an instance in which photosensitivity was prominent, and which is also of much interest because of coproporphyrinemia and the presence of large amounts of the two coproporphyrin isomers in the feces, without a definite increase in the urine. In this case, Grotepass and Defalque (33c) were able to achieve isolation only by collection and concentration on infusorial earth, over a long period of time. Taylor and

[4] Unpublished.

co-workers (91) have recently isolated both isomers I and III from a case of "chronic" porphyria in which the prominent clinical features were abdominal pain and skin lesions due to photosensitivity. As mentioned at the outset, the term porphyria connotes the formation of uroporphyrin, for which there was no evidence in either of these last two cases. At present the question cannot be answered as to whether such cases differ fundamentally from porphyria or represent simply an unusual variation of the disease. As noted in the following it is even more difficult to classify certain instances in which large excesses of coproporphyrin are excreted without co-existence of any of the usual symptoms of porphyria.

The separation of the two main clinical types of porphyria on the basis of isomer I or III excretion became still further complicated with the finding that the Waldenström porphyrin, which had come to be regarded as uroporphyrin III (11a; 60b; 99a, b, c) is actually a complex mixture of uroporphyrin I and of a type III porphyrin which may have seven rather than eight carboxyl groups (32c; 100q). The Waldenström type porphyrin has only been encountered in the urines of cases of the intermittent, acute porphyria and may undoubtedly be regarded as a distinctive feature of this form of the disease, although not as an isomer of uroporphyrin I.

Waldenström (99c) stated that in the acute type of porphyria little or no coproporphyrin was to be found in the urine and that for this reason he had not isolated it. In two cases in which uroporphyrin was isolated from the urine, Waldenström obtained coproporphyrin I from the feces. H. Fischer and Libowitzky (24j) had previously found both uro- and coproporphyrin I in the urine of a case of acute porphyria. In the cases studied by Mertens (60b) and Bingel (4a), the daily amount of uroporphyrin in the urine ranged from 50–100 mgm., while the coproporphyrin approximated 1 mgm. Recently Brunsting and Mason (10b) described a case of porphyria of mixed type characterized by epidermolysis bullosa, pigmentation of the skin and neuropathy. During one 24 hour period this patient excreted 11.4 mgm. of uroporphyrin and 2.0 mgm. of coproporphyrin. Uroporphyrin I was identified, but the isomer type of the coproporphyrin was not determined. While the concentration of coproporphyrin in the urine of cases of intermittent acute porphyria is thus comparatively slight, it usually suffices to permit isolation from relatively small volumes. Representative data from a number of cases are given in table 2. Actually a number of other cases studied exhibited equal or greater concentrations of coproporphyrin but in these instances it was not quantitated, the main attention being given to isolation of the Waldenström porphyrin (32c; 100q).

In certain cases, as for example no. 9 in table 2, the amount of coproporphyrin considerably exceeded that of uroporphyrin. The reason for this is not clear. The data in this case, over a considerable period of time, are shown in figure 4. As may be noted, this patient suffered a spontaneous relapse while in the hospital, for which no precipitating factor was ascertained. During this relapse it is seen that the urine was characterized by an increase of all of the abnormal

elements, including the porphobilinogen and porphobilin, uro- and coproporphyrins. It may be noted, too, that the amount of zinc complex was relatively larger during the relapse. The significance of the combination of uro- and coproporphyrin with zinc, is unknown. Günther (34a) recognized that the urinary porphyrins in cases of porphyria were often partly combined with a metal. Derrien and co-workers (15a, b) described zinc uroporphyrinuria and even ascribed a protective or detoxifying action of the zinc. So far as can be determined this suggestion has not received further attention. Watson and his associates (100k, o, q) have likewise observed the zinc complex of Walden-

TABLE 2

Relative amounts of copro- and uroporphyrin in cases of intermittent acute porphyria

PATIENT NUMBER	COPROPORPHYRIN IN γ/24 HRS	PREDOMINANT TYPE ISOMER	UROPORPHYRIN IN γ/24 HOURS
1	144	(III)	708
2	378	(III)	5,250
3	156	(I)	2,340
4	460	(I)	33,000
	554		147,000
	301		37,000
5	160		508
6	182		
	250		
	312		
	300		
7	307	(I, III)	
	406		34,000
8*	256		120
	240	(III)	
9 (see fig. 4)	401	(III)	160
	484		110
	2,582		412
	1,306		307
	150		75
10*	182	(III)	61

* Latent cases; mother and aunt, respectively, of case 9.

ström's uroporphyrin as a regular component of the urine in cases of intermittent acute porphyria. The data shown in figure 4 reveal that a considerable fraction of the coproporphyrin was likewise complexed with zinc. The possible contribution of soft glass to this complex was excluded by making the collection in Pyrex bottles only. The occurrence of the zinc complex of coproporphyrin is not peculiar to porphyria; Mertens (60a) observed it in the urine in cases of lead poisoning, a finding which we can confirm. Thus in one instance the 24 hour urine contained 1686 γ as the metal complex and 1107 γ as free coproporphyrin. The absorption spectrum and HCl numbers have repeatedly been noted to correspond with those of the zinc complex (24k; 100q).

Dobriner and co-workers (161) isolated a natural ester of coproporphyrin I from a case of congenital porphyria. This melted at 251°C. The HCl number was 0.3–0.5 per cent, from which concentration the compound was readily extracted by $CHCl_3$. While the esterifying group could not be identified, it was thought to be lipid in character. So far as can be determined this has not been encountered in other cases.

As noted in figure 4, porphobilinogen was present in the urine in association with uro- and coproporphyrin. The term porphobilinogen refers to a chromogen which exhibits an Ehrlich aldehyde reaction, and which is believed to be a precursor of uroporphyrin and porphobilin (99c, g). The latter is a brown pigment not possessing porphyrin characteristics and formerly referred to as "urofuscin"

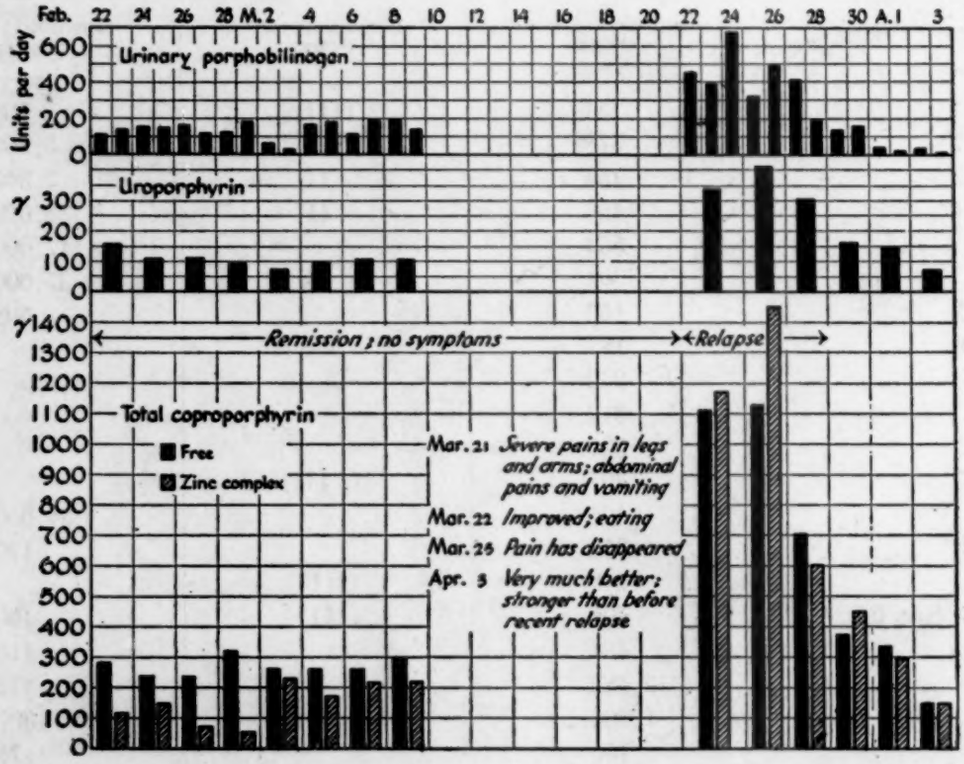

Fig. 4. Acute porphyria; excretion of porphobilinogen and of uro- and coproporphyrin, both free and zinc complex, during remission and relapse. M. ♀ C. 25 (case 9 in table 2).

(99c). It has long been known that the urines of cases of "acute" porphyria often exhibit an Ehrlich reaction, although whether due to urobilinogen or porphobilinogen was not determined in the past, since a method for differentiating these chromogens has only lately been described (99c; 100y). Insofar as the present subject is concerned, the question arises as to whether coproporphyrin is likewise excreted at least in part as a porphobilinogen. It is quite clear that even marked increases of urinary coproporphyrin are not associated with a porphobilinogen-Ehrlich reaction (100y), except in cases of porphyria in which uroporphyrin is also present. We have observed but two instances in which questionable reactions were encountered, and we have not found any proven examples in the literature. This does not, of course, exclude the possibility

that some of the coproporphyrin is excreted as a chromogen, but if so it does not have the behavior of porphobilinogen as found in the urine of porphyria cases, such as shown in figure 4.

The presence of large excesses of coproporphyrin, or of uroporphyrin, in the urine, is not correlated with the appearance of the corresponding porphyrins in the blood. In some instances uro- and/or coproporphyrin may be noted. Schumm (79a) observed uroporphyrin in a case of congenital porphyria in an approximate concentration of 1 mgm. per 100 cc. The urine in this case contained both uro- and coproporphyrin I. H. Fischer and Zerweck (24m) later reported the presence of coproporphyrin in the same case; in a separate report in the same year, however, they described uroporphyrinemia in this case (24o). In other cases of porphyria, both congenital (light sensitive) and intermittent acute in type, coproporphyrin has been noted in the serum (40; 78d; 96a; 96d). We have noted it repeatedly in 3 cases of the latter type, and have not been able to detect it in 6 others.

In one case the amount of coproporphyrin in the serum was determined to be 20 γ per 100 cc., while at the same time, the 24 hour urine sample contained 300 γ of coproporphyrin, most of which was type III. This sample also contained 1800 γ of uroporphyrin of the Waldenström type melting at 258°C. and separating on the $CaCO_3$ column into uroporphyrin I methyl ester (284°C.), and the 208° porphyrin (32c.).

The protoporphyrin of the erythrocytes has not been increased in several of our cases of intermittent acute porphyria, even during relapses of the disease when large amounts of uro- and coproporphyrin were found in the urine. Roth (75a) on the other hand studied a case in which the erythrocytes contained 74 γ of protoporphyrin per 100 cc., the urine 79 γ of coproporphyrin in a 24 hour sample. This was classified as an instance of "chronic" porphyria (Günther), since abdominal pain was associated with light sensitivity. The presence of diarrhea and hypochromic anemia suggest, however, that the moderate increase of the erythrocyte protoporphyrin was due, not to porphyria, but to iron deficiency (100p); the value for the urinary coproporphyrin was within normal limits.

The problem of excessive coproporphyrinuria without known cause is of much interest. For the time being at least, it is believed preferable to classify such cases, in which no uroporphyrin is found, as *idiopathic coproporphyrinuria*, rather than as porphyria, reserving the latter term to indicate a constitutional fault. This is in agreement with the separation emphasized by Waldenström (99c). At the same time it is recognized that certain cases excreting increased coproporphyrin, without uroporphyrin, may also represent "inborn errors of metabolism" (27d). A particularly interesting example of marked coproporphyrinuria without known cause has been studied recently (100w). This individual had no symptoms whatever, the excessive urinary coproporphyrin being noted in the course of a routine study of a large number of workers engaged in a special project. The urinary coproporphyrin was consistently in the range of 1–6 mgm. per day, practically all of which was the type III isomer. The

feces contained larger amounts of the same porphyrin, ranging up to 70 mgm. per 100 grams. None was detected in the blood at any time. It was readily established that the bile was rich in coproporphyrin III, but whether due to an enterohepatic circulation of porphyrin, could not be established. It appeared unlikely that an abnormal bacterial flora in the colon constituted an etiologic factor, since the bowel movements were normal and there were no symptoms referable to the intestinal tract. It was of interest that this man was a chemist who had worked in the past with heavy metals, especially lead and mercury. While there were no evidences of metal poisoning, the possibility was considered that a marked over production of coproporphyrin III may have been related to the effects of heavy metals or other chemicals (vide infra) in an individual of peculiar constitution.

Several cases have been studied (100x) in which intermittent abdominal pain was associated with moderate increases of urinary coproporphyrin, ranging from 120–600 γ per day, with an increased percentage of the type III isomer. Nothing was found in these cases to explain the increases. The fecal flora studied in certain of the cases did not appear abnormal. Nor were the symptoms or porphyrin excretion affected by a period on the Kämmerer diet (43b). This meat free diet is low in heme and porphyrin and was used by Kämmerer and Meyer in a similar case which they studied. Although they believed that the addition of liver or meat to this diet caused a significant increase of coproporphyrinuria, the data given are not convincing.

Barber and Howitt (1) reported several instances of photosensitivity in children, in which an increased fecal porphyrin was observed. It was not at all clear, however, that the porphyrin was in any way related to the photosensitivity. The type of porphyrin was not determined either in feces or urine and the statement that porphyrin was increased in the feces was based solely upon spectroscopic studies. Urbach (95a, b) also associated spasticity of the colon and dermatitis, especially solar eczema, with increased porphyrin formation in the bowel which was ascribed to an abnormal bacterial flora. Quantitative methods were not employed in this study. Alteration of the flora toward the normal, ascribed to elimination of animal protein from the diet, was said to result in marked improvement. Haranghy (36) reported an interesting case characterized by abdominal colic and circulatory disturbances. The urinary porphyrin was not increased but there was a marked increase in fecal porphyrin (type not mentioned). This was believed dependent on an abnormal flora which was almost wholly gram-negative It may be noted, however, that there were bleeding intestinal ulcers in the case, which may well have been significant from the standpoint of porphyrin formation.

The study of Reitlinger and Klee (72) is of interest with respect to the possible relation of coproporphyrin to abdominal colic, although it does not aid in determining the origin of the porphyrin. This study showed that very dilute solutions of coproporphyrin I instilled into exposed loops of rabbit intestine caused prolonged spastic contraction which was refractory to atropine.

The effect of chemicals and metals. In this group the effect of lead poisoning

was the first to receive study, increases in urinary porphyrin being observed by Garrod (27a, c) and Stokvis (86a) in human cases, and by Stokvis in experimental lead poisoning in rabbits (86a). The latter investigator believed that the porphyrin was derived from hemoglobin in the intestinal tract, which in turn was liberated by virtue of mucosal hemorrhages. In later studies (vide infra) it has been shown repeatedly that coproporphyrin III is the porphyrin which appears in the urine in excess, and, as noted previously, the evidence for a derivation of coproporphyrinuria III from blood in the intestinal tract is very dubious; furthermore, intestinal bleeding is not a feature of human lead poisoning. Stokvis' view has not been supported by any subsequent studies although it was subscribed to by Brugsch (9d). In several instances the feces have contained coproporphyrin I at a time when the urine yielded marked excesses of the type III isomer (98c; 100e).

Schumm (79b) was the first to identify coproporphyrin in the urine of cases of lead poisoning, and Grotepass (33b) first showed that it is the type III isomer, a finding which Fischer and Duesberg (24f) reported in the same year with respect to experimental lead poisoning in rabbits. Subsequent studies (16h; 60a; 98e; 100e) have extended and confirmed these observations with the result that coproporphyrinuria III has come to be recognized as a regular feature of lead intoxication. Günther's belief (34a) that this was of diagnostic value has been amply confirmed by later work (25c; 45b; 67; 78e) which has emphasized its value in industry. Franke and Litzner (25c) found a range of 12 to 181 γ per 100 cc. of urine in 43 workers, as compared with a normal of 3–7; 17 of the 43 had concentrations greater than 50 γ per 100 cc., and most of these exhibited other evidence of lead poisoning, including basophilic stippling. The experience of various investigators is shown in table 3.

The amounts noted in table 3 are insufficient, as a rule, to cause a red urine. This is, in fact, but rarely noted in lead poisoning. A case in which the urine was "port wine" color was observed by Crawford (14).

Knowledge of the effect of other metals on the urinary coproporphyrin is much more fragmentary. Considerable increases following mercury have been recorded by Vannotti (97b) and by Vigliani and Libowitzky (98c), but in both of these cases there were confusing complications, viz., a hematuria and hemoglobinuria in the former and a cirrhosis of the liver with chronic alcoholism in the latter. In Vannotti's case, there was light sensitivity and both uro- and coproporphyrin I were isolated, the daily output of the latter during the period of manifest illness ranging from 4 to 12 mgm. Two years later there was still light sensitivity and the coproporphyrin excretion ranged between 200–1300 γ per day. The uroporphyrin was found for a short time only during the acute poisoning and brings up the question as to whether this was a case of latent porphyria in which mercury was a precipitating factor.

Insofar as the case of Vigliani and Libowitzky is concerned, it has already been noted that coproporphyrin III is usually present in excess in cases of cirrhosis in chronic alcoholics, and thus it would be difficult to ascribe the excess in this case to mercury.

Hoerburger and Fink (39b) isolated coproporphyrin III from the urine of a case of salvarsan toxicity which had been studied by Schreus (78c). Subsequent studies (10c; 81) have revealed frequent significant increases in patients receiving salvarsan, more particularly in cases having exfoliative dermatitis (10c).

We have recently investigated a case of chronic arsenic poisoning characterized by typical hyperkeratoses, peripheral neuritis, and anemia. Arsenic was found in marked excess in the hair and urine, and, although the source was never proved, there was reason to believe that the patient had been poisoned intentionally. The urinary coproporphyrin excretion ranged from 1 to 3.2 mgm. per day. Since this patient had worked as a filling station attendant, at times siphoning tetraethyl lead gasoline and handling batteries, the possibility was considered that he also had lead poisoning. The presence of severe anemia and basophilic stippling were somewhat more suggestive of lead than of arsenic poisoning. The erythrocyte protoporphyrin in this case ranged from 200–400 γ

TABLE 3

Urinary coproporphyrin excretion in humans with lead poisoning

AUTHOR	PER DIEM EXCRETION*	PREDOMINANT ISOMER TYPE
Grotepass (33a)	To 8.0 milligrams	Copro-III
Schreus and Carrié (78e)	To 1.8 milligrams	Copro-III
Roth (75b)	To 1.4 milligrams	Not determined
Watson (100e)	To 1.0 milligrams	Copro-III
Vigliani and Angeleri (98b)	To 3.4 milligrams	Not determined
Mertens (60a)	To 5.0 milligrams	Not determined
Vigiliani and Libowitzky (98c)	To 2.0 milligrams	Copro-III
Vigliani and Waldenström (98e)	To 3.1 milligrams	Copro-III
Kark and Meiklejohn (45b)	To 1.2 milligrams	Not determined

* The values are not entirely comparable, as they are based in some instances on the yield of crystalline ester, and in others on fluorimetry.

per 100 cc. of erythrocytes, as compared with a normal range of 10–30 γ (100p). Data are not available with respect to the erythrocyte protoporphyrin in arsenic posisoning. Serum and urine lead determinations in this case were just at the upper limits of the normal ranges and were therefore not diagnostic.

Certain other investigations of the effects of metals on porphyrin excretion in animals may be mentioned. Thus Sumegi and Putnoky (89) observed that Pb, Hg, Bi, Cu, Zn, Fe, Au and Ag caused loss of weight in white rats, together with hyperfunction of the thyroid and porphyrinuria. Also mentioned were disturbances of liver and renal function. The intensity of these disturbances decreased in general in the order of the metals given. Thyroid hyperfunction and disturbance of porphyrin metabolism were greatest with Pb, Hg and Bi. These investigators found also that porphyrin alone, injected into rats, caused intense thyroid hyperfunction. Because of this they concluded that the porphyrin stimulated centers in the mid brain thus inducing a thyrotoxicosis. Obviously this study is of much interest and the results need to be confirmed.

Recently the effect on the urinary coproporphyrin of lead, uranium, thorium, beryllium, lanthanum, zirconium, mercury, and arsenic has been studied in both acute and chronic experiments on rabbits (80c). Insofar as lead is concerned the results of this study have confirmed those of previous investigators. Marked increases of type III isomer excretion, often persisting for a number of months, were noted to follow single injections of from 8–50 mgm. of lead (given as lead acetate) per kilo of body weight. Thorium, zirconium and lanthanum did not cause an increase except in animals which had received lead several months previously. Since this was associated with an increased excretion of lead, it was postulated that the mobilized lead rather than the newly administered metal of other type, was responsible for the increased coproporphyrin III. Uranium was found to cause a marked decrease in coproporphyrin excretion during the acute stage of poisoning. This was ascribed to renal injury. Smaller amounts were followed by a sustained, moderate increase. Injection of arsenic resulted in considerable increases of short duration.

A considerable literature has accumulated with respect to the relation of *sedatives* to porphyrin excretion and porphyria. The use of sulfonal and trional has often been followed by a disease which is indistinguishable from the intermittent acute porphyria of idiopathic type. Uroporphyrin has been isolated repeatedly from the urine. Ellinger and Riesser (19) noted a melting point of 255–257°C. which suggests a Waldenström type porphyrin such as usually encountered in acute porphyria; Dobriner, however, isolated uroporphyrin I from his case (16a). The findings in animal experiments have been somewhat confusing. Stokvis (86a) first noted an increased urinary porphyrin excretion in rabbits and dogs, following sulfonal. Fischer and Duesberg (24f) identified uroporphyrin spectroscopically, in urine from sulfonal poisoned rabbits. Waldenström and Wendt, in a later study (99d), found only coproporphyrin III. It was of interest in this study that dimethylsulfon-dimethylmethane, which differs from sulfonal only in having non-hypnotic methyl rather than hypnotic ethyl groups, was just as effective in increasing the urinary coproporphyrin. Neubauer (66) had been aware of the similar effect of both compounds many years earlier, although designating the porphyrin as hematoporphyrin. Waldenström (99c) has shown that trional can precipitate attacks of typical acute porphyria in members of the family of individuals in whom the disease had previously been diagnosed. This emphasizes the question, as yet not answered, whether sulfonal and trional porphyria represent merely a precipitation of a latent, constitutional disease.

Brugsch (9d, e) noted distinct, although not marked, increases of urinary coproporphyrin in patients receiving *paraldehyde*, *chloralhydrate*, *amylene hydrate*, and *morphine*, but not after therapeutic doses of *barbiturates*. According to Laubender and Monden (54) the urinary porphyrin excretion in rabbits is not increased by feeding of barbiturates. Duesberg (17b) and Haxthausen (38) both reported individual instances of acute porphyria following use of barbiturates. In the latter case phenobarbital had been taken, and it appeared that there was complete recovery following cessation of the drug. In Duesberg's

case uroporphyrin was tentatively identified. The possibility must be considered, as in the case of sulfonal and trional, that the barbiturate was only a precipitating factor in a latent, constitutional disorder. Eichler (18) and Brugsch (9d, e) believe that latent disturbance in porphyrin metabolism characterized only by mild nervous and mental disorders, may be exaggerated to the point of outspoken or fatal porphyria by the use of sedatives.

The effect of sulfonamides and other aromatic amines on the urinary coproporphyrin has been studied in some detail. Significant increases have often been observed following sulfanilamide administration to humans (73d; 84), especially where toxic reactions were encountered (10a, c; 21; 100m). Rimington and Hemmings (73c) noted marked increases in the urine and feces of rats receiving large amounts of sulfanilamide. This was true, also, with many other aromatic amines which they tested. Similar results were obtained by Brownlee (8a), who studied sulfanilamide, acetanilide, phenacetin, phenazone, amidopyrine, aspirin, and p-aminophenol. In both of these studies the excessive coproporphyrin was shown to be the type III isomer. It was postulated that this was derived from methemoglobin, the latter being formed by the oxidative action of the chemical (see Brownlee's paper (8a) for further discussion). This possibility will be considered later.

Penew and Tropp (68) reported increased urinary coproporphyrin excretion in humans following ether or nitrous oxide anesthesia, and in some instances after novocain (local) anesthesia. The increase in all of these cases was thought to be related to transitory liver injury, classified by them as a "serous inflammation." Tropp and Penew (93b) noted a large amount of coproporphyrin in the urine of an individual poisoned with illuminating gas. The isomer type was not mentioned in either of these papers.

The effect of carbon tetrachloride upon the urinary coproporphyrin has not been discussed in the literature. In a case of acute poisoning observed recently, a marked increase of the type III isomer in the urine was determined. This patient, however, was a moderate alcoholic so that the finding is somewhat more difficult to interpret. Thus it was found that the patient excreted 373 γ in 24 hours shortly after admission; 65 per cent of this was the type III isomer, as determined by the differential precipitation technique. Gillam and Kench (29) studied a case of severe methyl chloride poisoning characterized clinically by delirium, convulsions, and tremor. Marked increases of coproporphyrin III were isolated both from the urine and feces. A rapid decline in the amount occurred as the patient recovered. There was no anemia at any time.

The effect of *alcohol* on the urinary coproporphyrin has already been discussed in the section relating to liver disease, particularly the cirrhosis of chronic alcoholics.

Skin diseases and photosensitivity. The fundamental aspects of photosensitization by the porphyrins have been discussed in detail elsewhere (5a, b; 37b, c; 87a, b; 97c), and will not be considered here. In the main, outspoken photosensitivity has been a feature of congenital porphyria (uroporphyrin excretion) and has been noted but rarely in cases where coproporphyrin alone was observed

(vide infra). This is in accord with the observation of Fischer (24c) that in warm blooded animals, the degree of photosensitizing ability of the naturally occurring porphyrins is correlated with the number of carboxyl groups. Thus, uroporphyrin with eight is the most effective, coproporphyrin with four less so, and proto- and deuteroporphyrins with two each, not at all. On the other hand, the artificial hematoporphyrin, which also has but two carboxyl groups, is rather strongly light sensitizing both for man (24o; 61; 88) and animals (4b; 26; 28; 37b, c, b). Dobriner and Rhoads (16i) give the order of photosensitizing effect, as judged by the severity of "light shock" (24c) produced in white mice, as follows: "hematoporphyrin 9 (type III), uroporphyrin I, deuteroporphyrin 9 (type III), coproporphyrin I, and protoporphyrin 9 (type III). Coproporphyrin type III seems to be but slightly toxic and uroporphyrin 'type III' non toxic." Bingel (4b) also reported that coproporphyrin III is less sensitizing than I. Gildemeister (28) found that the uroporphyrins combine with the serum albumin fraction, but that coproporphyrins I and III do not. This is of obvious interest when it is remembered that blood serum has been reported to be protective against photosensitization (71; 82). Shibuya (82) found, however, that serum was also protective against the photosensitizing effect of coproporphyrin. Fischer and Zerweck (24m) had previously reported a protective action on the part of the brown pigment (urochrome) of normal and pathological urines, a compound which they believe was derived from protein. Mertens (60b) correlated the chemical findings in the urine with the presence or absence of photosensitivity, in the cases of porphyria reported up to the time of her paper (1937). All of these but two, i.e., the cases of van den Bergh (96d), and of Fischer and co-workers (24l), excreted uroporphyrin. The two exceptions, interestingly enough, both excreted coproporphyrin III, a finding in some conflict with the experimental results already referred to. As mentioned previously, the case of chronic porphyria recently reported by Taylor and co-workers (91) exhibited marked photosensitivity, yet the urine was said not to contain uroporphyrin. Both coproporphyrin isomers were identified. The case of Grotepass and Defalque (33c) appears to have been of the same type. The relation of coproporphyrin to the photosensitivity in these and other disease states is not at all clear. The case of idiopathic coproporphyrinuria cited earlier, in which unusually large amounts of the type III isomer were excreted, yet without manifestations of any kind, attests to the difficulty of the problem. Photosensitivity in certain skin diseases has been linked by certain investigators with a secondary coproporphyrinuria. Thus it has been suggested repeatedly that the dermatitis of pellagra is related to photosensitization by coproporphyrin (2; 3; 20; 59; 85). The methods used in these studies were not specific for porphyrin, and in fact it is clear that the color measured by the technique of Ellinger and Dojmi (20) is mainly due to formation of urorosein from indolacetic acid, rather than to porphyrin (100i, j, l). In alcoholic pellagra considerably increased amounts of coproporphyrin have been encountered in the urine (16k; 74; 100i). This has been identified as coproporphyrin III and has been noted to decline after nicotinic acid therapy and abstinence from alcohol (16k; 100i). As ob-

served earlier, however, this decline is quite usual in the ordinary alcoholic who stops drinking and resumes eating. Significant increases of coproporphyrin have not been found in endemic pellagra, either in urine (45a; 100i, l) or feces 100l). Rafsky (70) reported an increased porphyrinuria in aged subjects and ascribed it to dietary deficiency, but again the method employed was that of Ellinger and Dojmi (vide supra). The question of increased coproporphyrinuria in other diseases in which involvement of the skin is prominent has been reviewed by Dobriner (16i) who found conflicting reports concerning such conditions as erythema multiforme, lupus erythematosus, and xeroderma pigmentosum. Considerable variability was also evident in the rather extensive study of Brunsting and co-workers (10c). It may be noted with respect to this study that of nine cases of so called "eczema or urticaria solare", only two exhibited increases of porphyrin excretion and these were but slight. Goeckermann, Osterberg and Sheard (30) had previously noted a considerable increase in one such instance. Thus it appears that, as in hydroa aestivale seu vacciniforme, the porphyrins are implicated in some but not all cases of solar eczema or urticaria.

Origin and significance. It may be well in concluding this review to attempt to correlate what has gone before in terms of the more fundamental aspects of the coproporphyrins, i.e., their site of formation and functions. The evidence, however, is too scanty to permit conclusions and it is only possible to summarize the existing knowledge and theories.

It is clear that the coproporphyrins are easily synthesized from "niedrige Bausteine" by unicellular organisms, viz., yeast cells (24g, k, n), and various types of bacteria (11; 13; 42; 57). In this connection, it is of interest that yeast cells undergoing autolysis manufacture coproporphyrin I without reducing their formation of protoporphyrin 9 (type III) (24g). Bacteria, on the other hand, synthesize coproporphyrin III (42; 53a, b), at least insofar as is known at present. The significance of this difference has not been determined. Whether the synthesis of coproporphyrin is as easily achieved by animal cells is unknown. Recent studies have shown that protoporphyrin, at least, is readily formed from such simple precursors as acetates and glycine (4c; 81a). The transition to coproporphyrin is theoretically rather easy, requiring merely the addition of formic acid to the vinyl groups of protoporphyrin (24l). van den Bergh and his co-workers (96c) believed they were able to prove the occurrence of this transition in liver perfusion experiments, but their results could not be confirmed by Watson and co-workers (77; 100n).

Borst and Königsdörffer (7) observed coproporphyrin in the megaloblasts of embryonic bone marrow and of the bone marrow in the case Petry in whom pernicious anemia was said to have been important in the cause of death. The subsequent finding of an increased excretion of coproporphyrin I in pernicious anemia (16h, g, l; 98c; 100f), hemolytic anemias (16a, d, m; 100b), and regenerative post hemorrhagic anemias (16h), all of which are characterized by increased, albeit differing varieties of erythropoiesis in the bone marrow, led to postulation that formation of coproporphyrin I is proportional to the rate of

erythropoiesis (16d, h, m; 100f). Rimington (73e) and Dobriner and Rhoads (16g) advanced the theory that coproporphyrin I is formed as a by-product of hemoglobin synthesis, Rimington (73e) suggesting a normal ratio of 10,000 of type III (protoporphyrin) to 1 of type I (coproporphyrin). The constant proportion in rate of formation of these two porphyrins noted by Dobriner and Rhoads led them to suggest, quite similarly, that "the two substances are not synthesized independently but are products of the same chemical process". As shown in figure 5, they postulated that two dipyrrylmethenes, A and B, are the precursors of both types I and III porphyrins. The main synthesis proceeding to the protoporphyrin 9 of the hemoglobin molecule would thus depend upon coupling

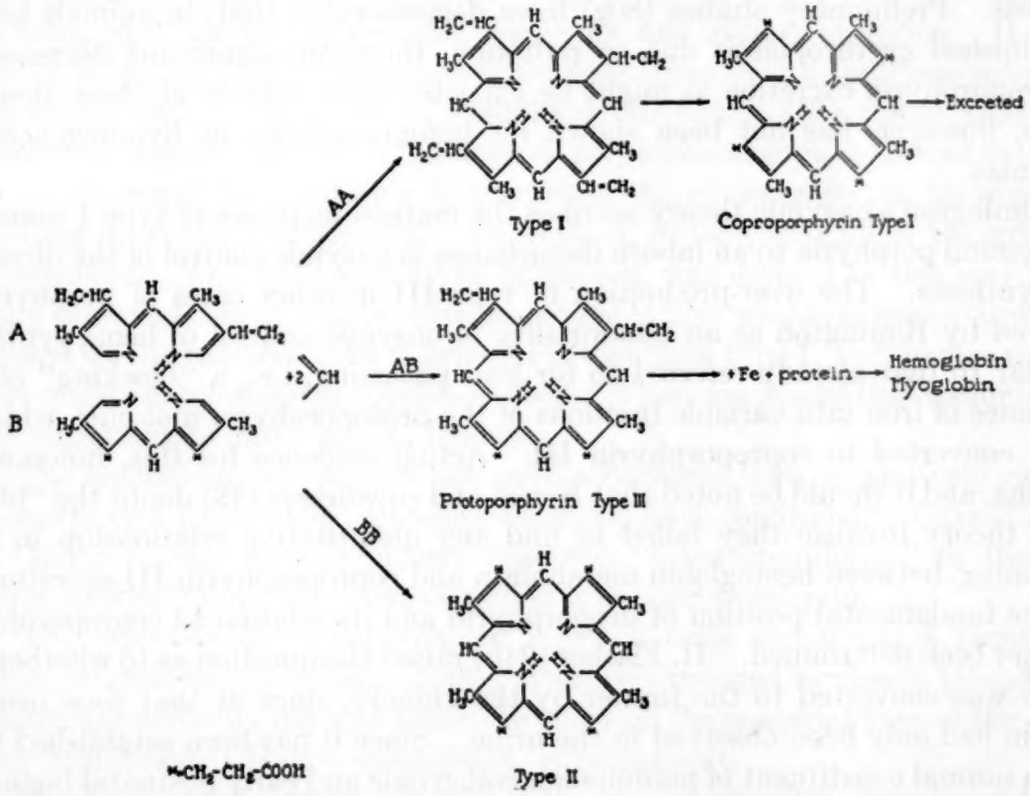

Fig. 5. Scheme of formation of hemoglobin and the coproporphyrins as according to Dobriner and Rhoads, J. Clin. Investigation 17: 95, 1935. Reproduced by permission of the publishers.

of two molecules A and B while the formation of coproporphyrin I would depend upon coupling of two molecules of A. It is further seen that two molecules of B would form a type II porphyrin. As already noted, however, there is no evidence for the occurrence of the latter type in nature. The scheme shown in figure 5 does not attempt to answer the question as to whether coproporphyrin I is formed by direct synthesis or by addition of formic acid to the vinyl groups of an isomeric (type I) protoporphyrin. According to Rimington's theory (73e) iron is "blocked" by certain substances, such as lead, from combination with protoporphyrin, and the latter, which is thus not used in hemoglobin synthesis, is converted to coproporphyrin III. It should be noted that this is regarded as a

disturbance in "Aufbau" rather than "Abbau". The latter, however, would be represented by the transition: hemoglobin → methemoglobin → coproporphyrin III, which Rimington and Hemmings (73c) and Brownlee (8b) believe to be the mode of elaboration induced by the sulfonamides and similar chemicals. Rimington's theory assumes that porphyrin formation is under an enzymic control, the nature of which is such that under normal circumstances, the above mentioned ratio of 10,000 to 1 prevails. According to this, in quite the same manner as postulated by Rhoads and Dobriner, an increased excretion of coproporphyrin I would be expected in conditions associated with increased erythropoiesis. Further studies of fecal and urinary coproporphyrin excretion are needed in a variety of anemias having both increased and decreased erythropoiesis. Preliminary studies (80g) have demonstrated that, in animals having diminished erythropoiesis due to radiation, there are significant decreases in coproporphyrin excretion as might be expected on the basis of these theories. This, however, has not been shown for human aplastic or hyporegenerative anemias.

Rimington's enzymic theory ascribes the marked increases of type I isomer in congenital porphyria to an inborn disturbance in enzymic control of the direction of synthesis. The over-production of type III in other cases of porphyria is viewed by Rimington as an abnormality in enzymic control of heme synthesis similar to that already referred to for lead poisoning, i.e., a "blocking" of the entrance of iron into variable fractions of the protoporphyrin molecule, which is then converted to coproporphyrin III. Actual evidence for this, however, is lacking, and it should be noted that Kench and co-workers (48) doubt the "blocking" theory because they failed to find any quantitative relationship in lead poisoning, between hemoglobin metabolism and coproporphyrin III excretion.

The fundamental position of uroporphyrin and its relation to coproporphyrin has not been determined. H. Fischer (24b) raised the question as to whether the latter was converted to the former by the kidneys, since at that time uroporphyrin had only been observed in the urine. Since it has been established that it is a normal constituent of mammalian embryonic and early postnatal bone and that it is still present in the ossicles of the inner ear in later postnatal life (51a), further, that in porphyria it is commonly encountered in the feces (80a; 100q) and in the viscera, especially the liver (7; 24q; 100q), the important question for future studies to answer is whether coproporphyrin formation proceeds over uroporphyrin by simple decarboxylation. While uroporphyrin probably has some function in pre- and early postnatal life, the possibility exists that under normal circumstances in later postnatal life, it is formed and rapidly decarboxylated to coproporphyrin. Porphyria, on this basis, would consist of a marked over production of uroporphyrin with varying degrees of failure of decarboxylation to coproporphyrin, in different cases.

The possibility also exists, however, that the essential constitutional error in porphyria is a further carboxylation of copro- to uroporphyrin. This would have to include, of course, an excessive production of coproporphyrin and a variable efficiency of carboxylation in order to explain cases of the type repre-

sented in figure 4. From a strictly chemical standpoint, the primary synthesis of uroporphyrin with secondary decarboxylation would appear to be the more likely of the two possibilities. This needs further study as a possible normal mechanism of coproporphyrin formation.

The possibility of formation of a fraction of the urinary coproporphyrin in the intestinal tract either from hemoglobin, or as a product of bacterial metabolism, at least under certain circumstances, cannot be wholly excluded. Yet it can be said that the contribution from either of these sources is at least quite small and that the urinary coproporphyrin is mainly endogenous. It is probable that coproporphyrin III is formed in small amount both from blood in the intestine and by certain of the intestinal flora, but the most recent objective studies (53a, b) have failed to reveal any appreciable absorption and urinary excretion. It may be noted, however, that the discrepancy between these results and those reported by Kaunitz (46), is unexplained. Mallinckrodt-Haupt (57) stated that the increased coproporphyrin (I) of the urine in pernicious anemia is derived entirely from an abnormal bacterial flora in the colon, the abnormality, in his opinion, depending upon the achlorhydria. He implied, in fact, that all of the urinary coproporphyrin, whether normally or in disease, was derived in this way. As already observed, the available evidence indicates that whatever coproporphyrin is formed by the bacterial flora is type III rather than type I, and further that it is very questionable whether this is absorbed. There are in addition, a number of other objections to this view, as follows:

1. The meconium, which does not have a bacterial flora is relatively rich in coproporphyrin I (79b; 99f), a finding which we have confirmed.[5] Garrod (27c) and Günther (34a) had previously observed "hematoporphyrin" in the meconium, and Schumm (79b) first identified it as coproporphyrin.

2. The excretion of coproporphyrin I in the feces and urine of pernicious anemia patients decreases significantly after liver induced remission (16e; 100d) although the achlorhydria and hence the effect on the bacterial flora of the colon which Mallinckrodt-Haupt emphasizes, is permanent.

3. The amounts of coproporphyrin I in the feces of hemolytic jaundice patients usually exceed considerably those of pernicious anemia in relapse (100d, e, f), yet the gastric hydrochloric acid and the bacterial flora of the colon in this disease are, usually at least, quite normal.

4. If the bacterial flora of the intestine were mainly responsible for the urinary coproporphyrin, one would have to assume a differing flora in such conditions as aplastic or refractory anemia on the one hand from that in infectious hepatitis on the other, or in infectious hepatitis from that in poliomyelitis, in accordance with the recent findings as referred to in the foregoing. While such a difference is barely possible, it would appear to be quite unlikely.

With the possible exception of a derivation of coproporphyrin III from methemoglobin, as discussed previously, there is little evidence for, and a great deal against the catabolic theory of coproporphyrin formation. It appears that

[5] Unpublished.

Schreus (78b) was the last to champion this belief, although without offering any definite support for it. The earlier investigators, viz., Stokvis, Garrod, Saillet, Günther and others had rather naturally assumed such an origin since, as previously pointed out, they had all confused copro- with hematoporphyrin, and the derivation of the latter from hemoglobin, in vitro, was one of the few things about the porphyrins that was clearly appreciated in that period. Schreus (78b, c, g), and Carrié (11), although well aware of the nature of the coproporphyrins, believed that they were derived from hemoglobin destruction principally because of the speed of increase in the urine after onset of the disease process, and because of their data indicating a proportionality between rate of blood destruction and amount of coproporphyrin excreted. Insofar as the type I isomer is concerned, it is clear that this could be related just as well to the rate of erythropoiesis, as emphasized by the theories of Rimington and Dobriner (vide supra). Furthermore, the data of others (48; 100e) have not revealed correlation between the rate of hemoglobin catabolism and the excretion of coproporphyrin III either in urine or feces.

Klüver's discovery (51a, b) of the regular occurrence of small amounts of coproporphyrin in the central nervous system of warm blooded animals, and the tentative identification as the type III isomer together with the increased excretion of this isomer in poliomyelitis, lead one to question whether the urinary porphyrin may be derived at least in part from that of the nervous system. The different orders of magnitude of the concentrations in the brain and the urine speak against this possibility, but perhaps do not exclude it. Nothing is known of the rate of formation of coproporphyrin, and if Granick and Gilder's hypothesis (31) is correct, that coproporphyrin may serve as a regulator of oxygen consumption by living cells, it would be conceivable that nervous tissue might produce and liberate it much more rapidly under certain circumstances. As yet, nothing is known as to possible alterations of central nervous system coproporphyrin concentration in disease.

Prior to Klüver's discovery, Keilin (47) had suggested that cytochrome might be the parent substance of coproporphyrin. Klüver (51b) pointed out, however, that the areas relative'y rich in cytochrome were poor in coproporphyrin, or devoid of it entirely, and vice versa.

Reasons have been given in the foregoing for ascribing the increased urinary coproporphyrin I in hepatitis and many cases of cirrhosis, to hepato-cellular functional impairment. It is impressive, however, that a chronic alcoholic with advanced cirrhosis and obvious liver functional impairment, usually does not excrete increased type I, but rather a considerable amount of the type III isomer. One may contrast two cases of cirrhosis exhibiting about equally severe disturbance of hepatic function in other respects, one, a chronic alcoholic excreting 150 γ of type III and but 50 of type I, the other, a non-alcoholic whose cirrhosis followed an infectious hepatitis, excreting 175 γ of type I and 15 γ of type III. This suggests that various disease agents drive or direct the coproporphyrin formation one way or the other, and that there may be a closer relationship between the formation of the two isomers than hitherto considered.

Further studies should seek to determine whether there is a balanced production of the coproporphyrins normally favoring type I, and whether disturbances in this ratio are subject in part to constitutional factors. The latter appear requisite to explain the striking differences thus far encountered in such well defined entities as Hodgkin's disease and hemochromatosis. At the same time it is recognized that other and as yet unknown factors may be responsible.

Thus the exact site and mode of formation of the coproporphyrins and their physiological rôle, remain in doubt. It would appear that microfluorospectrometry and the use of synthetic porphyrins containing C_{14}, are of most promise for the immediate future investigation of these questions.

REFERENCES

(1) Barber, H. W. and Z. Howitt. Guy's Hosp. Rep. **76:** 314, 1926.
(2) Bassi, U. Clin. Med. et al. **65:** 241, 1934.
(3) Beckh, W., P. Ellinger and T. D. Spies. Quart. J. Med. **30:** 305, 1937.
(4a) Bingel, A. Fortschr. d. Neurol. **9:** 265, 1937.
(b) Bingel, A. Ztschr. f. d. ges. Neurol. u. Psychiat. **158:** 79, 1937.
(c) Block, K. E. and D. Rittenberg. J. Biol. Chem. **159:** 45, 1945.
(5a) Blum, H. F. Photodynamic action and diseases caused by light. New York, Reinhold Publishing Corp., 1941.
(b) Blum, H. F. and H. J. Templeton. J. A. M. A. **108:** 548, 1937.
(6) Boas, I. Deutsch. med. Wchnschr. **4:** 126, 1933.
(7) Borst, M. and H. Königsdorffer. Untersuchungen über Porphyrie. S. Hirzel, Leipzig, 1929.
(8a) Brownlee, G. Proc. Roy. Soc. Med. **32:** 1276, 1939.
(b) Brownlee, G. Biochem. J. **33:**697, 1939.
(9a) Brugsch, J. T. Ztschr. f. d. ges. exper. Med. **95:** 471, 1935.
(b) Brugsch, J. T. Ztschr. f. d. ges. exper. Med. **95:** 482, 1935.
(c) Brugsch, J. T. Ergebn. d. ges. Med. **20:** 424, 1935.
(d) Brugsch, J. T. Ergebn. d. inn. Med. u. Kinderh. **51:** 86, 1936.
(e) Brugsch, J. T. Ztschr. f. physiol. Chem. **99:** 585, 1936.
(f) Brugsch, J. T. and A. Keys. Proc. Staff. Meetings Mayo Clinics **12:** 609, 1937.
(g) Brugsch, J. T. Mensch u. Chlorophyll; Ergeb. d. inn. Med. u. Kinderheilk. **56:** 614, 1939.
(10a) Brunsting, L. A. Proc. Staff Meetings Mayo Clinic **12:** 614, 1937.
(b) Brunsting, L. A. and H. L. Mason. J. A. M. A. **132:** 509, 1946.
(c) Brunsting, L. A., J. T. Brugsch and P. A. O'Leary. Arch. Dermatol. and Syph. **39:** 294, 1939.
(11) Carrié, C. Die Porphyrine. G. Thieme, Liepzig, 1936.
(11a) Chandler, F. G., C. A. Harrison and C. Rimington. Brit. Med. J. **2:** 1173, 1939.
(b) Cartwight, G. E., M. A. Lauritsen, P. J. Jones, I. M. Nerrill and M. M. Wintrobe. J. Clin. Investigation **25:** 65, 1946.
(12) Chu, E. J. H. and C. J. Watson. To be published.
(13) Coulter, C. B. and F. M. Stone. Proc. Soc. Exper. Biol. and Med. **38:** 423, 1938.
(14) Crawford, H. Med. J. Australia **1:** 589, 1933.
(15a) Derrien, E. and C. Benoit. Bull. Soc. Chim. Fr. **43:** 522, 1928.
(b) Derrien, E. and P. Cristol. C. R. Soc. Biol. Paris **103:** 126, 1930.
(16a) Dobriner, K. J. Biol. Chem. **113:** 1, 1936.
(b) Dobriner, K. Proc. Soc. Exper. Biol. and Med. **35:** 175, 1936.
(c) Dobriner, K. J. Biol. Chem. **120:** 115, 1937.
(d) Dobriner, K. Proc. Soc. Exper. Biol. and Med. **36:** 757, 1937.
(e) Dobriner, K. and W. H. Barker. Proc. Soc. Exper. Biol. and Med. **36:** 864, 1937.

(f) DOBRINER, K. AND C. P. RHOADS. New England Med. J. **219:** 1027, 1938.
(g) DOBRINER, K. AND C. P. RHOADS. J. Clin. Investigation **17:** 95, 1938.
(h) DOBRINER, K. AND C. P. RHOADS. J. Clin. Investigation **17:** 105, 1938.
(i) DOBRINER, K. AND C. P. RHOADS. Physiol. Rev. **20:** 416, 1940.
(j) DOBRINER, K., C. P. RHOADS AND L. E. HUMMEL. J. Clin. Investigation **17:** 125, 1938.
(k) DOBRINER, K., W. H. STRAIN AND S. A. LOCALIO. Proc. Soc. Exper. Biol. and Med. **38:** 748, 1938.
(l) DOBRINER, K., W. H. STRAIN, H. GUILD AND S. A. LOCALIO. J. Clin. Investigation **17:** 761, 1938.
(m) DOBRINER, K., W. H. STRAIN, S. A. LOCALIO, H. KEUTMANN AND D. I. STEPHENS. Proc. Soc. Exper. Biol. and Med. **36:** 755, 1937.
(n) DOBRINER, K., W. H. STRAIN, AND S. A. LOCALIO. Proc. Soc. Exper. Biol. and Med. **36:** 752, 1937.
(17a) DUESBERG, R. Arch. f. exper. Path. u. Pharmakol. **162:** 249, 1931.
(b) DUESBERG, R. München med. Wchnschr. **79:** 1821, 1932.
(18) EICHLER, P. Ztschr. Neurol. **141:** 363, 1932.
(19) ELLINGER, A. AND O. RIESSER. Ztschr. f. physiol. Chem. **98:** 1, 1916.
(20) ELLINGER, P. AND F. DOJMI. Chem. and Industry **13:** 507, 1935.
(21) FIGGE, F. H. G., T. H. CAREY AND G. S. WEILAND. J. Lab. and Clin. Med. **31:** 752, 1946.
(22) FIKENTSCHER, R. Biochem. Ztschr. **249:** 257, 1932.
(23) FINK, H. AND W. HOERBURGER. Die Naturwissenschaften **18:** 292, 1934.
(24a) FISCHER, H. Ztschr. f. physiol. Chem. **95:** 34, 1915.
(b) FISCHER, H. Ztschr. f. physiol. Chem. **96:** 148, 1915.
(c) FISCHER, H. Ztschr. f. physiol. Chem. **97:** 109, 1916.
(d) FISCHER, H. Ztschr. f. physiol. Chem. **155:** 96, 1926.
(e) FISCHER, H. Oppenheimer's Handb. d. Biochem. des Menschen und die Tiere. Zweite Auflage, Ergänzungsband p. 72, G. Fischer, Jena, 1930.
(f) FISCHER, H. AND E. DUESBERG. Arch. f. exper. Path. u. Pharmakol. **166:** 95, 1932.
(g) FISCHER, H. AND H. FINK. Ztschr. f. physiol. Chem. **150:** 243, 1925.
(h) FISCHER, H. AND H. HILMER. Ztschr. f. physiol. Chem. **153:** 167, 1926.
(i) FISCHER, H. AND J. HIERNEIS. Ztschr. f. physiol. Chem. **196:** 155, 1931.
(j) FISCHER, H. AND LIBOWITZKY. Ztschr. f. physiol. Chem. **241:** 220, 1936.
(k) FISCHER, H. AND H. ORTH. Die Chemie des Pyrrols. Bd II; I Hälfte, Leipzig: Akad. Verlagsgesellsch., 1937.
(l) FISCHER, H., K. PLATZ AND K. MORGENROTH. Ztschr. f. physiol. Chem. **182:** 265, 1929.
(m) FISCHER, H. AND W. ZERWECK. Ztschr. f. physiol. Chem. **132:** 12, 1924.
(n) FISCHER, H. AND K. SCHNELLER. Ztschr. f. physiol. Chem. **135:** 253, 1924.
(o) FISCHER, H. AND W. ZERWECK. Ztschr. f. physiol. Chem. **137:** 176, 1924.
(p) FISCHER, H. AND K. ZEILE. Liebig's Ann. d. Chem. **468:** 98, 1929.
(q) FISCHER, H., H. HILMER, F. LINDNER AND B. PÜTZER. Ztschr. f. physiol. Chem. **150:** 44, 1925.
(25a) FRANKE, K. Ztschr. f. klin. Med. **130:** 222, 1936.
(b) FRANKE, K. AND R. FIKENTSCHER. München med. Wchnschr. **82:** 171, 1935.
(c) FRANKE, K. AND S. LITZNER. Ztschr. f. klin. Med. **129:** 115, 1935.
(26) GAFFRON, H. Ztschr. f. physiol. Chem. **179:** 157, 1926.
(27a) GARROD, A. E. J. Physiol. **13:** 598, 1892.
(b) GARROD, A. E. J. Path. and Bact. **1:** 87, 1893.
(c) GARROD, A. E. Lancet **2:** 1323, 1900.
(d) GARROD, A. E. Inborn errors of metabolism. H. Froude, London 1923. 2nd ed.
(28) GILDEMEISTER, H. Ztschr. f. d. ges. exper. Med. **102:** 58, 1937.
(29) GILLAM, A. E. AND J. E. KENCH. Lancet, London **2:** 806, 1940.

(30) GOECKERMAN, H. W., A. E. OSTERBERG AND C. SHEARD. Arch. Dermat. and Syph. **20:** 501, 1929.
(31) GRANICK, S. AND H. GILDER. Science **101:** 540, 1945.
(32a) GRINSTEIN, M. La Prensa Medica **44:** 2241, 1940.
(b) GRINSTEIN, M. La Prensa Medica **45:** 2295, 1940.
(c) GRINSTEIN, M., S. SCHWARTZ AND C. J. WATSON. J. Biol. Chem. **157:** 323, 1945.
(d) GRINSTEIN, M. Revista Arg.—Norteamic. de Cienc Med. **9:** 2, 1944.
(33a) GROTEPASS, W. Ztschr. f. physiol. Chem. **205:** 193, 1932.
(b) GROTEPASS, W. Ztschr. f. physiol. Chem. **253:** 276, 1938.
(c) GROTEPASS, W. AND A. DEFALQUE. Ztschr. f. physiol. Chem. **252:** 155, 1938.
(34a) GÜNTHER, H. Ergebn. allg. Path. u. path. Anat. **20:** 608, 1922.
(b) GÜNTHER, H. Krankheiten des Blutes und der blutbildenden Organe. Bd 2, Berlin: Julius Springer, 1925.
(35) HALTER, K. Arch. f. Dermatol. u. Syph. **178:** 340, 1938.
(36) HARANGHY, L. Centralbl. f. allg. Path. u. path. Anat. **54:** 161, 1932.
(37a) HAUSMANN, W. Wien. klin. Wchnschr. **22:** 1820, 1909.
(b) HAUSMANN, W. Sonderbande z. Strahlentherapie. Wien-Berlin: Urban and Schwarzenberg, 1923.
(c) HAUSMANN, W. AND H. HAXTHAUSEN. Die Lichtkrankungen der Haut. Urban and Schwarzenberg; Berlin and Vienna, 1929.
(d) HAUSMANN, W. AND F. M. KUEN. Biochem. Ztschr. **265:** 105, 1933.
(38) HAXTHAUSEN, H. Dermat. Wchnschr. **84:** 827, 1927.
(38a) HILL, R. AND H. F. HOLDEN. Biochem. J. **20:** 1326, 1926.
(39a) HOERBURGER, W. Inaugural Dissertation, Erlangen, 1933.
(b) HOERBURGER, W. AND H. FINK. Ztschr. f. physiol. Chem. **236:** 136, 1935.
(40) HOESCH, K. AND C. CARRIÉ. Ztschr. f. klin. Med. **129:** 214, 1935.
(41) HOPPE-SEYLER, F. Cited by NENCKI AND SIEBER (64).
(42) JAKOB, A. Klin. Wchnschr. **18:** 1024, 1939.
(43a) KÄMMERER, H. Klin Wchnschr. **2:** 1153, 1923.
(b) KÄMMERER, H. AND W. K. MEYER. Deutsch. Arch. f. klin. Med. **179:** 392, 1936.
(44a) KAPP, E. M. AND A. F. COBURN. Brit. J. Exper. Path. **17:** 255, 1936.
(b) KAPP, E. M. Brit. J. Exper. Path. **20:** 33, 1939.
(45a) KARK, R. AND A. P. MEIKLEJOHN. Am. J. Med. Sc. **201:** 380, 1941.
(b) KARK, R. AND A. P. MEIKLEJOHN. J. Clin. Investigation **21:** 91, 1942.
(46) KAUNITZ, H. Ztschr. f. klin. Med. **133:** 552, 1938.
(47) KEILIN, D. Proc. Roy. Soc. London (B) **98:** 312, 1925.
(48) KENCH, J. E., A. E. GILLAM AND R. E. LANE. Biochem. J. **36:** 384, 1942.
(49) KEYS, A. AND J. BRUGSCH. J. Am. Chem. Soc. **60:** 2135, 1938.
(50) KIRSTAHLER, A. Tab. Biologicae Periodicae **7:** 48, 1931.
(51a) KLÜVER, H. J. Psychol. **17:** 209, 1944.
(b) KLÜVER, H. Science **99:** 482, 1944.
(c) KLÜVER, H. Personal communication.
(52) LAGEDER, K. Arch. f. Verdauungskr. **56:** 237, 1934.
(53a) LARSON, E. A. Graduate Thesis—Univ. of Minn., November 1946.
(b) LARSON, E. A. AND C. J. WATSON. Studies of Coproporphyrin (V) (to be published).
(54) LAUBENDER, W. AND K. MONDEN. Arch. f. exper. Path. u. Pharmakol. **188:** 562, 1938.
(55) LIBOWITZKY, H. AND K. F. SCHEID. Klin. Wchnschr. **17:** 156, 1938.
(56) MACMUNN, C. A. Proc. Roy. Soc. of London **31:** 206, 1880.
(57) v. MALLINCKRODT-HAUPT, A. S. Klin. Wchnschr. **20:** 190, 1941.
(58a) MASON, H. L. AND S. NESBITT. J. Biol. Chem. **152:** 19, 1944.
(b) MASON, V. R., C. COURVILLE AND E. ZISKIND. Medicine **12:** 355, 1933.
(59) MASSA, M. Riforma med. **48:** 1669, 1932.

(60a) MERTENS, E. Klin. Wchnschr. **16:** 61, 1936.
(b) MERTENS, E. Ztschr. f. physiol. Chem. **250:** 57, 1937.
(61) MEYER-BETZ, F. Deutsch. Arch. f. klin. Med. **112:** 476, 1913.
(62) MICHELI, F. AND G. DOMINICI. Deutsch. Arch. f. klin. Med. **171:** 154, 1931.
(63) MINIBECK, H. Biol. Ztschr. **293:** 219, 1937.
(64) NENCKI, M. AND N. SIEBER. Arch. f. exp. Path. u. Pharmakol. **24:** 430, 1888.
(65) NESBITT, S. AND A. M. SNELL. Arch. Int. Med. **69:** 573, 1942.
(66) NEUBAUER, O. Arch. f. exper. Path. u. Pharmakol. **43:** 456, 1900.
(67) OTTO, H. Arch. f. Gewerbepath. u. Hyg. **8:** 655, 1938.
(68) PENEW, L. AND C. TROPP. Deutsch. Arch. f. klin. Med. **180:** 423, 1937.
(69) POULIKAKOS, P. AND C. TROPP. Deutsch. Arch. f. klin. Med. **183:** 342, 1938.
(70) RAFSKY, H. A. AND B. NEWMAN. Am. J. Digest. Dis. **9:** 43, 1942.
(71) RASK, E. N. AND W. H. HOWELL. Am. J. Physiol. **84:** 363, 1928.
(72) REITLINGER, K. AND P. KLEE. Arch. f. exper. Path. u. Pharmakol. **127:** 277, 1938.
(73a) RIMINGTON, C. Onderstepoort J. Vet. Sc. **7:** 567, 1936.
(b) RIMINGTON, C. Ann. Rev. Biochem. **12:** 425, 1943.
(c) RIMINGTON, C. AND A. W. HEMMINGS. Lancet **1:** 770, 1938.
(d) RIMINGTON, C. AND A. W. HEMMINGS. Biochem. J. **33:** 960, 1939.
(e) RIMINGTON, C. C. r. Lab. Carlsberg, Sér. chim. **22:** 454, 1938.
(74) ROSENBLUM, L. A. AND N. JOLLIFFE. Am. J. Med. Sc. **199:** 853, 1940.
(75a) ROTH, E. Deutsch. Arch. f. klin. Med. **178:** 185, 1935.
(b) ROTH, E. Ztschr. f. klin. Med. **129:** 123, 1936.
(76) SAILLET. Rev. de. méd. **16:** 542, 1896.
(77) SALZBURG, P. AND C. J. WATSON. J. Biol. Chem. **139:** 593, 1941.
(78a) SCHREUS, H. T. Klin. Wchnschr. **4:** 121, 1934.
(b) SCHREUS, H. T. Klin. Wchnschr. **13:** 121, 1934
(c) SCHREUS, H. T. Klin. Wchnschr. **14:** 1717, 1935.
(d) SCHREUS, H. T. AND C. CARRIÉ. Dermat. Ztschr. **62:** 357, 1931.
(e) SCHREUS, H. T. AND C. CARRIÉ. Ztschr. f. klin. Med. **125:** 330, 1935.
(f) SCHREUS, H. T. AND C. CARRIÉ. Klin. Wchnschr. **11:** 1017, 1931.
(g) SCHREUS, H. T. Klin. Wchnschr. **13:** 334, 1934.
(79a) SCHUMM, O. Ztschr. f. physiol. Chem. **105:** 168, 1919.
(b) SCHUMM, O. Ztschr. f. physiol. Chem. **126:** 169, 1923.
(c) SCHUMM, O. Arch. f. exper. Path. u. Pharmakol. **191:** 529, 1939.
(d) SCHUMM, O. Ztschr. f. d. ges. exper. Med. **106:** 252, 1939.
(80a) SCHWARTZ, S. AND C. J. WATSON. Proc. Soc. Exper. Biol. and Med. **47:** 390, 1941.
(b) SCHWARTZ, S., V. E. HAWKINSON AND C. J. WATSON. Science **103:** 338, 1946.
(c) SCHWARTZ, S. AND R. ZAGARIA. Plutonium Project Record Vol. 22, B (to be published).
(d) SCHWARTZ, S. Plut. Proj. Record, Vol. 20 (to be published).
(e) SCHWARTZ, S., V. E. HAWKINSON, S. COHEN AND C. J. WATSON. J. Biol. Chem. **168:** 133, 1947.
(f) SCHWARTZ, S., R. HUNTER, M. GLICKMAN AND J. WALLACE. Plut. Proj. Record Vol. 22 (to be published).
(g) SCHWARTZ, S., R. ZAGARIA AND C. J. WATSON. Plutonium Proj. R., v. 22 (to be published).
(81) SCOLARI, E. Giorn. Ital. Dermatol. Sifilog. **78:** 5, 1937.
(81a) SHEMIN, D. AND D. RITTENBERG. J. Biol. Chem. **159:** 567, 1945.
(82) SHIBUYA, H. Strahlentherapie **17:** 412, 1924.
(83) SIEDEL, W. Die Chemie **56:** 169, 1943.
(84) SILVER, B. AND M. ELLIOTT. J. A. M. A. **112:** 723, 1939.
(85) SPIES, T. D., Y. SASAKI AND E. GROSS. South. Med. J. **31:** 483, 1938.
(86a) STOKVIS, B. J. Ztschr. f. klin. Med. **28:** 1, 1895.
(b) STOKVIS, B. J. Ned. Tijdschr. Geneesk. **13:** 413, 1889.

(87a) STOKES, J. H., H. BEERMAN AND N. R. INGRAHAM. Am. J. Med. Sc. **203:** 608, 1942.
(b) STOKES, J. H., H. BEERMAN AND N. R. INGRAHAM. Am. J. Med. Sc. **204:** 601, 1942.
(88) STRAUCH, C. B. Am. J. Dis. Child. **40:** 800, 1930.
(89) SUMEGI, S. AND J. PUTNOKY. Arch. f. Gewerbepath. u. Hyg. **9:** 566, 1939.
(90) SUTHERLAND, D., V. E. HAWKINSON AND C. J. WATSON. Studies of Coproporphyrin (IV) (to be published).
(91) TAYLOR, J. J., M. L. SOLOMON, G. S. WIELAND AND F. J. FIGGE. J.A.M.A. **131:** 26, 1946.
(92a) THIEL, W. Verh. deutsch. Ges. inn. Med. 81, 1933.
(b) THIEL, W. Klin. Wchnschr. **19:** 1, 1934.
(93a) TROPP, C. AND K. SIEGLER. Deutsch. Arch. f. klin Med. **180:** 402, 1937.
(b) TROPP, C. AND L. PENEW. Deutsch. Arch. f. klin Med. **180:** 411, 1937.
(94) TURNER, W. J. J. Lab. and Clin. Med. **26:** 323, 1940.
(95a) URBACH, E. Klin. Wchnschr. **17:** 304, 1938.
(b) URBACH, E. AND J. BLÖCH. Wien. klin. Wchnschr. **47:** 527, 1934.
(96a) VAN DEN BERGH, H. AND W. GROTEPASS. Klin. Wchnschr. **15:** 586, 1933.
(b) VAN DEN BERGH, H. AND M. HYMAN. Deutsch. med. Wchnschr. **36:** 1492, 1928.
(c) VAN DEN BERGH, H., W. GROTEPASS AND F. E. REVERS. Klin. Wchnschr. **11:** 1534, 1932.
(d) VAN DEN BERGH, H., R. REGNIERS AND W. MULLER. Arch. f. Verdauungskr. **42:** 302, 1928.
(97a) VANNOTTI, A. Ergebn. d. inn. Med. u. Kinderh. **49:** 337, 1935.
(b) VANNOTTI, A. Arch f. gewerbepath. u. Hyg. **8:** 266, 1937.
(c) VANNOTTI, A. Porphyrine und Porphyrinkrankheiten. J. Springer, Berlin, 1937.
(98a) VIGLIANI, E. C. Arch. per le Sc. Med. **55:** 391, 1938.
(b) VIGLIANI, E. C. AND C. ANGELERI. Klin. Wchnschr. **15:** 700, 1936.
(c) VIGLIANI, E. C. AND H. LIBOWITSKY. Klin. Wchnschr. **16:** 1243, 1937.
(d) VIGLIANI, E. C. AND B. SONZINI. Arch. per le Sc. Med. **65:** 363, 1938.
(e) VIGLIANI, E. C. AND J. WALDENSTRÖM. Deutsch. Arch. f. klin. Med. **180:** 182, 1937.
(99a) WALDENSTRÖM, J. Acta Med. Scandinav. **83:** 281, 1934.
(b) WALDENSTRÖM, J. Deutsch. Arch. f. klin Med. **178:** 38, 1935.
(c) WALDENSTRÖM, J. Acta Med. Scandinav. 1937, Supp. 82.
(d) WALDENSTRÖM, J. AND S. WENDT. Ztschr. f. physiol. Chem. **259:** 157, 1939.
(e) WALDENSTRÖM, J., H. FINK AND W. HOEBURGER. Ztschr. f. physiol. Chem. **233:** 1, 1935.
(f) WALDENSTRÖM, J. Ztschr. f. physiol. Chem. **239:** Supp. III, 1936.
(g) WALDENSTRÖM, J. AND B. VAHLQUIST. Ztschr. f. physiol. Chem. **260:** 189, 1939.
(100 a) WATSON, C. J. Arch. Int. Med. **47:** 698, 1931.
(b) WATSON, C. J. J. Clin. Investigation **14:** 106, 1935.
(c) WATSON, C. J. J. Clin. Investigation **14:** 110, 1935.
(d) WATSON, C. J. J. Clin. Investigation **14:** 116, 1935.
(e) WATSON, C. J. J. Clin. Investigation **15:** 327, 1936.
(f) WATSON, C. J. J. Clin. Investigation **16:** 383, 1937.
(g) WATSON, C. J. Downey's Handbook of hematology, Vol. IV. Paul Hoeber, New York, 1938.
(h) WATSON, C. J. Oxford Medicine **4:** 228, 1938.
(i) WATSON, C. J. Proc. Soc. Exper. Biol. and Med. **39:** 514, 1938.
(j) WATSON, C. J. Proc. Soc. Exper. Biol. and Med. **41:** 591, 1939.
(k) WATSON, C. J. South. Med. J. **36:** 1, 1943.
(l) WATSON, C. J. AND J. A. LAYNE. Ann. Int. Med. **19:** 183, 1943.
(m) WATSON, C. J. AND S. SCHWARTZ. Proc. Soc. Exper. Biol. and Med. **44:** 7, 1940.
(n) WATSON, C. J., L. J. PASS AND S. SCHWARTZ. J. Biol. Chem. **139:** 583, 1941.
(o) WATSON, C. J. AND S. SCHWARTZ. Abstract. Proc. Soc. Clin. Investigation, May, 1941.

(p) WATSON, C. J., M. GRINSTEIN AND V. E. HAWKINSON. J. Clin. Investigation **23**: 69, 1944.
(q) WATSON, C. J., S. SCHWARTZ AND V. E. HAWKINSON. J. Biol. Chem. **157**: 345, 1945.
(r) WATSON, C. J., W. SCHULZE, V. E. HAWKINSON AND A. B. BAKER. Proc. Soc. Exper. Biol. and Med. **64**: 73, 1947.
(s) WATSON, C. J., S. SCHWARTZ, D. SUTHERLAND AND V. E. HAWKINSON. Studies of Coproporphyrin (I) (to be published).
(t) WATSON, C. J., R. C. CAPPS, E. M. RAPPAPORT AND V. E. HAWKINSON. Studies of Coproporphyrin (II) (to be published).
(u) WATSON, C. J., D. SUTHERLAND AND V. E. HAWKINSON. Studies of Coproporphyrin (III) (to be published).
(v) WATSON, C. J. AND V. E. HAWKINSON. Studies of Coproporphyrin (VI) (to be published).
(w) WATSON, C. J., R. ZAGARIA, B. MORGAN AND S. SCHWARTZ. Studies of Coproporphyrin (VII) (to be published).
(x) WATSON, C. J. AND V. E. HAWKINSON. Studies of Coproporphyrin (VIII) (to be published).
(y) WATSON, C. J. AND S. SCHWARTZ. Proc. Soc. Exper. Biol. and Med. **47**: 393, 1941.
(101) WEISS, H. Deutsch. Arch. f. klin. Med. **149**: 255, 1925.